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CHANGES IN THE BLOOD AFTER MUSCULAR ACTIVITY AND DURING TRAINING

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Some years ago a number of investigators made a study of the changes wrought in the blood of man by muscular work. The majority of these determined only the changes in the number of the red or white corpuscles or in the specific gravity immediately at the close of the exercise and at one later period. In this study we have endeavored to follow the sequence of the changes in the blood's content of erythrocytes, total leucocytes, variety of leucocytes, platelets, and haemoglobin, and also the variations in the specific gravity, from the moment the exertion ended until equilibrium was again attained; and to find an explanation of these changes. In addition we have examined the blood of several athletes throughout a period of training to determine the effects of regular and frequently repeated muscular work.

The routine followed in each of our work experiments comprised a preliminary determination of the normal number of the several varieties of blood corpuscles, the percentage of haemoglobin, and the specific gravity of the blood. After this the subject either ran eight-tenths of a mile around a part of the college campus, or made a rapid run of 260 yards in a build-

ing in the course of which he ascended three flights of stairs, or worked for 15 or 20 minutes on a stationary bicycle. In several experiments the subject ran two miles on a cinder track. Immediately at the close of the exercise blood samples were taken from a finger so punctured that the blood flowed freely. At intervals of 15 minutes, or thereabout, other samples were drawn from fresh punctures until it was found that the normal condition of the blood was reëstablished. For these observations we had nine subjects and made thirty-six experiments.

The percentage of haemoglobin was determined with the Gower-Haldane haemoglobinometer and the counts of blood corpuscles were made with the Thoma-Zeiss haemocytometer. The red corpuscles were diluted with Hayem's solution, the white corpuscles with a 1 per cent solution of acetic acid, and the platelets with Wright and Kinnicutt's mixture of cresyl blue and potassium cyanide. In making the differential counts of the leucocytes Wright's triple stain was employed and two or three hundred of the corpuscles counted in at least two spreads of blood. For the specific gravity a series of wide-mouthed bottles containing mixtures of glycerine and water of different densities was employed.

PERCENTAGE OF CHANGES IN THE BLOOD

An increase in the number of red corpuscles occurs during muscular exertion. Willebrand (1) found this increase to vary between 2.9 and 23.4 per cent. Zuntz and Schumburg (2) in a study of German soldiers during marches obtained increases of 247,000 to 650,000 per cubic millimetre or an average increase of 9 per cent. Hawk (3) in his study of college athletes found the increase in the number of red corpuscles to vary from 400,000 to 1,460,000 or from 7.3 to 26.7 per cent. The minimum rise in the erythrocytes in our own experiments was 200,000 and the maximum 1,180,000. The percentage of increase varied between 3.2 and 22.8. The increase in the number of red corpuscles for a given exertion was not constant. One of our subjects ran eight-tenths of a mile twelve times,

the concentration in red corpuscles varied between 3.2 and 11.1 per cent. We believe these differences find an explanation in the distribution of the blood as determined by previous activity and stage of digestion.

The changes in haemoglobin, on the whole, were proportionate, but not always equal, to the increase in the number of the erythrocytes. The increase in our subjects varied between 3.5 and 10.9 per cent The maximum rise in haemoglogin was scarcely half the maximum for the red corpuscles. The parallelism was decidedly lacking in two experiments and in these the red corpuscles were far in excess. These two experiments gave our maximum figures for the rise in erythrocytes.

Hawk cites three investigations in which a leucocytosis was found after muscular work, the number of leucocytes varied from 11,400 to 22,200. His own observations on athletes showed the increase in the number of leucocytes to vary from 1,930 to 9,500 per cubic millimetre or from 21 to 104.4 per cent. Zuntz and Schumburg in their study of soldiers obtained an average leucocytosis of 43 per cent or an increase of 2,470 to 3,080 over the number found when the men were at rest. We, likewise, have found, following exercise, a corresponding increase in the number of white corpuscles; this increase varied from 1,170 to 11,670 while the percentage ranged from 13.8 to 130.2.

The influence of muscular activity upon the several kinds of leucocytes has been considered by Zuntz and Schumburg. They obtained an increase of polymorphonuclears of from 6 to 11 per cent and a decrease in the lymphocytes of from 3 to 17 per cent. Burrows (4), on the other hand, in a study of a single case found that exercise decreased the polymorphonuclear elements and increased the lymphocytes. Our own differential counts are invariably in accord with those of Zuntz and Schumburg and show an increase of from 9 to 45 per cent in polymorphonuclear elements and a decrease of from 14 to 55 per cent in the lymphocytes. Burrows found that convulsions increased the polymorphonuclears and decreased the lymphocytes and attempted to establish the fact that these changes were not due

to muscular activity but to a pathological condition. Our records correspond closely with the changes he found in these cases of convulsions and indicate that the blood changes observed by him after the convulsions may have been wholly due to the muscular activity of the attack.

We find no record of observations on the blood platelets following exercise. There was in our experiments a marked fall in the number per cubic millimetre, shortly after the cessation of muscular action, of from 17 to 30 per cent. This was later followed by an increase which carried the number above the normal 17 and more per cent.

The specific gravity of the blood in muscular activity has been carefully studied by Jones (5). He found that it usually varied directly with the red corpuscles. However, he reports that gentle exercise is accompanied by a fall, while the more prolonged or violent forms of exercise are accompanied by a rise in the specific gravity. Zuntz and Schumburg constantly obtained an increase in the specific gravity of the blood. The exercises used by us invariably caused a rise.

THE SEQUENCE IN THE CHANGES

We were unable to follow in every experiment all of the blood changes in which we were interested; nevertheless, in the large number of experiments made we have studied each change many times and in considerable detail. On several occasions the observations covered the six factors. The results of one of these experiments are given in the curves of figure 1. These show the sequence of the alterations in the blood as they were found to occur following a run of eight-tenths of a mile. The curves bring out rather clearly the fact that the changes in the number of erythrocytes, percentage of haemoglobin, and specific gravity are more or less parallel and suggest that they may be the result of a common causative factor. The curves showing changes in the number of leucocytes, lymphocytes, and platelets are placed together because of an association to be shown later.

The immediate effect of the exercises used was a concentration of the blood of the peripheral capillaries. Within a very few minutes after the completion of the exercise the blood began to

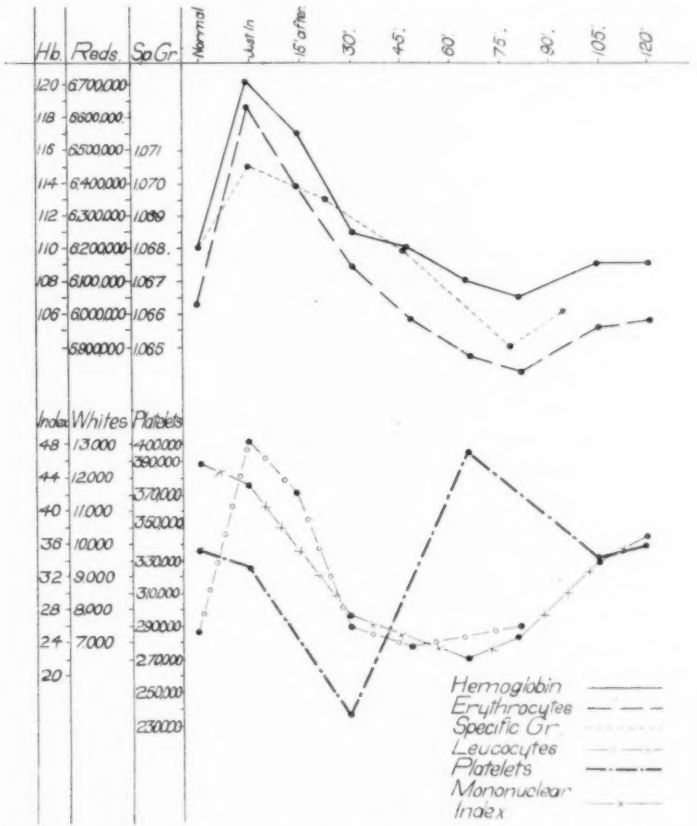

Fig. 1. The blood changes following a run of eight-tenths of a mile in 4.2 minutes.

dilute, and as a result the percentage of haemoglobin, the number of red corpuscles, and the density gradually diminished so that each returned to its normal figure in from 30 minutes to an hour and a quarter. The time required seemingly was

dependent upon the length of the period of muscular activity. However, the return to the normal condition of the blood was only temporary, the diluting process so continued that the composition with respect to haemoglobin, erythrocytes, and specific gravity soon became subnormal. The entire diluting process lasted from 50 minutes to 2 hours. After this the blood again concentrated and soon restored to the normal the three factors. The entire series of post-exercise changes was usually completed within 1 or 2 hours.

The maximum and minimum figures for the variations in haemoglobin, red corpuscles, and specific gravity in the various experiments showed some differences. In a number of instances the haemoglogin rose above and fell below the normal by an equal amount; in others the degree of dilution was less than the amount of concentration; while in four instances the subnormal stage did not appear. The percentage of change for haemoglobin and the number of red corpuscles was sometimes the same; for example, maximum haemoglobin 3.4, erythrocytes 3.2, minimum haemoglobin 3.4, and erythrocytes 3. Very frequently the percentage of change for the number of red corpuscles was greater than that for the haemoglobin as the following illustrates: maximum of haemoglobin 5.5 and of red corpuscles 6.9, minimum of former 5 and of latter 8, and another in which both the maximum and minimum for haemoglobin were 4.2 and for the red corpuscles 9.1.

The specific gravity varied directly with the red corpuscles, so that having determined the normal for both and the change in either the number of corpuscles or the specific gravity the amount of change for the other could be fairly closely predicted. The specific gravity variations were all similar to the following: (1) normal 1.068, maximum after exercise 1.0705, minimum 1.066; (2) normal 1.070, maximum 1.0715, minimum 1.069.

The form of the curve showing the sequence of the variations in the number of the leucocytes per cubic millimetre of blood does not correspond with those for haemoglobin, erythrocytes, and specific gravity. The percentage increase in leucocytes at the end of the exertion was usually far greater than it was

for these other factors and this gives a higher apex to the plotted curve. Usually the return to the normal number of white corpuscles was more abrupt, and occasionally slower, than was the case for haemoglobin or red corpuscles. Instances in which the changes were parallel were exceptional. The leucocyte count rarely showed a subnormal stage.

Differential counts of the leucocytes indicate a slow destruction of the lymphocytic elements of the blood following muscular work. Immediately at the close of the exertion there was, as a rule, no definite change in the proportions of the various kinds

TABLE 1

Differential counts after an eight-tenths mile run

	POLYNUCLEARS	SMALL LYMPHOCYTES	LARGE LYMPHOCYTES	LARGE MONONUCLEARS	TRANSITIONALS	EOSINOPHILES	MAST	INDEX
Normal	51.0	22.0	18.0	4.0	2.0	1.0	2.0	46.0
Just in	53.5	20.5	17.0	4.5	1.5	1.0	2.0	43.5
32 minutes	69.5	19.0	7.0	1.5	0	0	2.0	27.5
66 minutes	74.0	11.5	7.5	3.0	0.5	0	1.5	22.5
105 minutes	73.0	9.0	13.0	1.0	2.0	0	2.0	25.0
135 minutes	60.0	17.0	13.0	2.0	2.0	0	1.0	34.0
168 minutes	62.0	16.5	14.0	6.0	0.5	0.5	1.0	37.0
4 hours	50.0	32.0	10.0	5.0	1.0	1.0	1.0	48.0

of leucocytes. Slowly thereafter throughout a period of from 1 to 2 hours the polymorphonuclear elements increased and the lymphocyte index, which is obtained by adding the total number of mononuclears, the large and small lymphocytes, large mononuclears, and transitionals, decreased. The details of these changes as they were found in a typical experiment are given in Table I. The lymphocytic index was found to fall from 23 to 52 per cent. The following are examples of the total change in the index: 41.5 to 20, 39 to 26, and 42 to 26. The return to the normal proportions of the kinds of leucocytes was also gradual, requiring a period of from 2 to 4 hours. Dr. G. B. Gilbert informs us that the return to the normal lympho-

cyte index may be delayed as much as 24 hours after a night of dancing. The change in the proportion of the several varieties of leucocytes can not be accounted for by an influx of certain kinds from the tissues during exertion, since although the total number of leucocytes is enormously increased during the exercise the differential counts were, at the immediate close of work, either only very slightly or not at all altered. Further the changes in the differential counts continued long after the normal number of leucocytes per cubic millimetre had been restored.

The changes in the number of the blood platelets did not correspond to those of the other blood elements studied. Immediately at the close of muscular work, as was the case with the lymphocytes, it was found that there was either no change or a very slight decrease in the number. However, there then followed, apparently as a consequence of a destructive process, a rapid fall in the number which reduced the platelets within 30 to 60 minutes from approximately 350,000 to 250,000 per cubic millimetre. After this came a period of rapid increase which resulted in an over-production. Hence within an hour or two after exertion the number exceeded the normal from 17 to 25 per cent. Later as is shown in figure 1 there was a return to the normal number.

Hawk found in his study of various kinds of college athletic activities that the increase in the number of red corpuscles in the blood became gradually less pronounced as the exercise was continued. This he believed pointed toward the possibility of an actual decrease due to a rapid destruction of red corpuscles in prolonged violent exercise. We have five observations bearing on this point in which the subjects had walked a distance of 9.7 miles to the top of Pike's Peak, ascending approximately 7,900 feet. The average grade was 1 in 6 or 7, with some half dozen stretches of considerable length with a 25 per cent grade. The ascent was made in from 4 to 5 hours. It should be here noted that each man drank as much water as he desired during the climb. For three of the men only haemoglobin determinations were made, of these on arrival at the summit one showed

a drop from 112, his normal in Colorado Springs, to 108 or a fall of 3.6 per cent; the second gave no change, his normal 112 being obtained; the third had an increase from 111 to 116 or 4.5 per cent. Both the haemoglobin and the number of red corpuscles were determined for the other two subjects. One gave a haemoglobin reading of 116 and had 6,514,000 red corpuscles per cubic millimetre in Colorado Springs, while upon arrival at the summit the haemoglobin was 127, or concentrated 9.5 per cent, and the red corpuscles 6,920,000, an increase of 16.2 per cent. The fifth man had in Colorado Springs a haemoglobin content of 112 and 6,224,000 red corpuscles, arriving on Pike's Peak these had increased to 128 or 14.3 per cent and to 6,872,000 or 10.4 per cent respectively. The after-diluting process which soon produces a subnormal condition, occurred in each subject but could not be followed to the end because of lack of proper light for titrating the blood. In the last two subjects observations throughout a period of 4 hours failed to show the process of dilution completed.

These results, taken as a whole, do not support Hawk's theory that there is an increased destruction of erythrocytes during muscular activity. The work accomplished was severe and long continued and thus gave ample time for destruction of corpuscles. Nevertheless, in only one of the five men was there a suggestion of such destruction. Our subject who showed the lowered content of haemoglobin was known to be the least fit physically. Circulatory observations, such as arterial, capillary, and venous pressures, and rate of blood flow, proved that he did not react as well as physically strong men. Evidence, which is given later, suggests that the decrease in the haemoglobin and red corpuscles was not due to an actual destruction of corpuscles but rather to their stagnation in some of the capillaries because of fatigue.

Zuntz and Schumburg found the blood concentrated in soldiers who had marched for seven hours over a course of 24.75 kilometres carrying loads of from 27 to 31 kilos.

THE EXPLANATION OF THE BLOOD CHANGES

Hawk advances six possible explanations for the increase of erythrocytes in exercise. These are (1) the production of new corpuscles, (2) concentration of the blood through increased urine formation and copious sweating, (3) concentration of the blood through increased evaporation in the lungs, (4) passage of fluid from the blood to the active muscles, (5) concentration of the blood through vaso-motor contraction and rise in blood pressure, and (6) sudden passage into the blood of a large number of cells lying dormant in various parts of the body. He is inclined to accept the last factor as the primary cause of the increase in red corpuscles since in a short run, such as a hundred yard dash, involving only a few seconds in time there would not be sufficient opportunity for any of the first five factors to accomplish the degree of concentration observed. As exercise is continued the factors of copious sweating, accelerated urine formation, increased evaporation from the lungs, and the passage of fluid from the blood to the working muscle, all help to keep up the concentrated condition of the blood and so obscure his predicated opposite action, namely an accelerated destruction of red corpuscles. Willebrand believes that the withdrawal of water from the blood by the working muscles is the primary cause of the concentration. Zuntz and Schumburg accept Willebrand's explanation.

To account for the leucocytosis found to be present after muscular activity, Hawk adds another to the six factors mentioned above; it is that there is a changed distribution of the leucocytes followed by their accumulation in the peripheral circulation. This he believes is the primary cause of leucocytosis, which he further explains by the fact that greatly increased rapidity of circulation would throw out from the interior into the general circulation a great number of leucocytes which arriving in the peripheral arterioles and venules would lag behind the general blood stream and produce an apparent leucocytosis of large dimensions. Zuntz and Schumburg believe since the white corpuscles increase so much more than the red that

a different explanation must obtain for their increase. They hold that the passing of wandering cells from the tissues into the general circulation is an adequate explanation.

We have attempted in several series of experiments to determine the importance of the various explanations offered for the changes in the blood during exertion. That there is a stagnation of red corpuscles which withdraws large numbers from the general circulation is indicated in the following observation. In this experiment the subject ran eight-tenths of a mile in 4 minutes and 50 seconds. His blood was then examined at intervals until found to be well down in the subnormal stage when he once more ran, in $5\frac{1}{2}$ minutes, the same distance. The results follow:

	HAEMOGLOBIN	RED CORPUSCLES
	per cent	*millions*
Normal	117	6.4
1 minute after first run	125	7.2
23 minutes after first run	119	
35 minutes after first run	115	6.2
57 minutes after first run	114	
60 minutes ran second time		
1 minute after second run	123	7.1
17 minutes after second run	118	6.5
30 minutes after second run	116	
47 minutes after second run	111	5.9
83 minutes after second run	111	5.9
120 minutes after second run	112	

As a result of the first run the haemoglobin content had increased 6.8 and the number of red corpuscles 12.5 per cent; in the second the haemoglobin again rose above the normal 5.1 and the red corpuscles 10.9 per cent, while they were 7.9 and 14.5 per cent respectively above their content at the start of the second run. The return of both, during the second run, to almost the values obtained after the first makes it probable that we are not here dealing with a loss of water from the body but only with a side-tracking of a large mass of corpuscles.

Abdominal pressure experiments. Mitchell (6) reported that in a healthy subject general massage increased the number of

red corpuscles and to a lesser degree, or sometimes not at all, the haemoglobin. He obtained in a case of chronic lead poisoning an increase in the erythrocytes from 4,000,000 to 4,500,000 with the application of abdominal massage for 25 minutes. This observation points to the splanchnic area as a large source of reserve corpuscles and led us to test the influence of abdominal massage. It early became apparent that prolonged abdominal massage was not necessary to bring about the rise, so instead a heavy weight of lead foil, weighing 25 pounds and so shaped as to fit between the lower ribs and the top of the pelvis, was placed on the abdomen of the reclining

TABLE 2

Weight-massage of the abdomen

SUBJECT	TIME	HAEMOGLOBIN	ERYTHROCYTES	LEUCOCYTES
L.C.H.	Normal	115	6,328,000	9,600
	5 minutes	120	6,876,000	10,200
	20 minutes	119	6,856,000	10,000
E.C.S.	Normal	106	5,240,000	8,000
	4 minutes	108	5,432,000	8,400
	16 minutes	110	5,640,000	8,300
D.L.S.	Normal	116	6,392,000	
	4 minutes	119	6,688,000	
	19 minutes	123	6,976,000	

subject. The act of breathing moved the weight slightly and gave a massage effect with the result that in 2 or 3 minutes an increase in haemoglobin and red corpuscles was evident in the peripheral circulation. Such experiments were prolonged 10 to 30 minutes. Frequently the maximum change in the blood occurred within 5 minutes but in others was delayed as much as 20 minutes. We used six subjects and each several times for these observations. The data for three typical experiments are given in table 2. The average increase for all of our observations was haemoglobin 5.1 and red corpuscles 8.7 per cent. These figures are strikingly close to the average of the increases obtained from all of our work experiments in which the average increase in haemoglobin was 6.3 and in red corpuscles 9.6 per

cent. Immediately after a meal and while digestion was at its height abdominal pressure failed to alter the blood.

Prevention of return to normal. If the splanchnic area is the chief reservoir of reserve red corpuscles it may be that pressure on the abdomen after exertion, by delaying side-tracking, will prevent the return to the normal content of haemoglobin and erythrocytes. To test this two methods were employed, the first, which was the least effective, was to draw a belt around the abdomen as tightly as possible within three minutes after the return from a run; the second was to have the subject take the reclining posture with the lead weight on the abdomen. Both methods were always effective in preventing to a considerable degree the dilution. Thus in one experiment by use of the tight belt with the subject standing, after a run of eight-tenths of a mile had increased the haemoglobin from 113 to 118 and the red corpuscles from 5,840,000 to 6,560,000, at the end of an hour and a half the haemoglobin was still 115 and the erythrocyte count 6,144,000. A protocol of an experiment for which the subject ran a half mile in 3 minutes and 58 seconds and afterward reclined with a weight resting on his abdomen follows:

	HAEMOGLOBIN	RED CORPUSCLES
Normal	107	5,680,000
Just in	115	6,360,000
19 minutes later	114	
39 minutes later	113	6,328,000
61 minutes later	111	6,240,000
85 minutes later	111	6,100,000
23 minutes after the weight was removed	107	5,696,000

Curves have been plotted for this experiment in figure 2 and placed in contrast with curves from a typical experiment when the diluting process was not interfered with. The curve for the typical reaction returns to the normal level and passes into the subnormal phase in about 50 minutes, while that expressing the condition in which abdominal pressure was employed only descends half way to the normal in 85 minutes. It is improbable that either method used for preventing the stagnation of

blood in the splanchic area is completely effective hence we conclude that the splanchnic area is the primary source of the reserve of red corpuscles thrown into the general circulation during muscular activity.

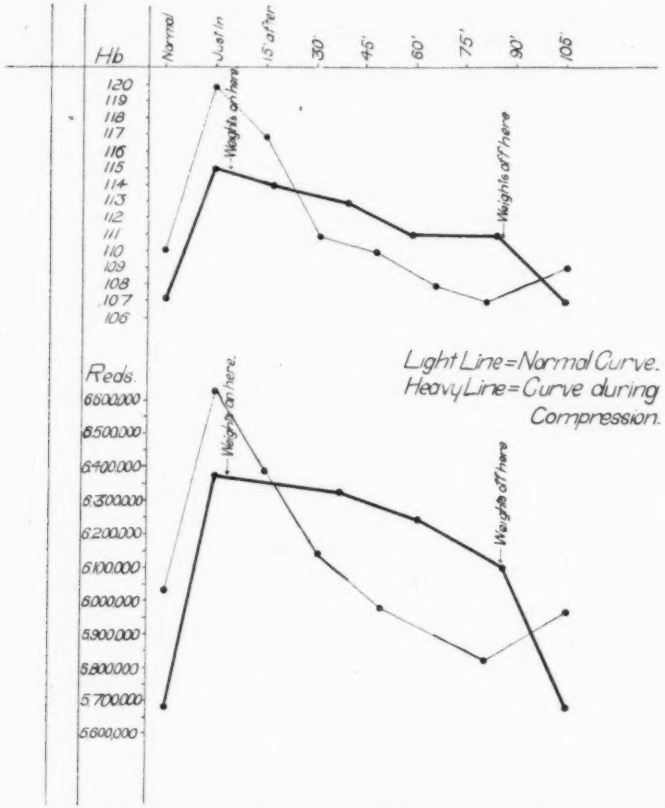

Fig. 2. A comparison of the changes in the haemoglobin and red corpuscles after runs of eight-tenths of a mile following which the abdomen was in one instance compressed and in the other not under pressure.

Work and abdominal pressure experiments at a high altitude. Two series of observations made upon the summit of Pike's Peak, on subjects who had sojourned there from 3 to 10

days, strengthen the above conclusions. In one series six subjects did muscular work either by making a run of 175 yards up a path of considerable grade, a part being a 25 per cent incline, or ran about a half mile on the level, or worked for about 15 minutes on a stationary bicycle. In eleven experiments the customary rise in haemoglobin, red corpuscles, and specific gravity did not occur. While in a majority immediately at the close of exertion the blood had not changed, in three instances it had diluted 2 and more per cent. In each work experiment the post-diluting process appeared as at the lower altitude. A typical protocol for a half mile run is given below:

	HAEMOGLOBIN	ERYTHROCYTES	SPECIFIC GRAVITY	PLATELETS	LEUCOCYTES
		millions			
Normal	126	6.9	1.0665	360,000	6,500
Just in	126	6.9	1.0660		10,200
30 minutes later	124	6.6	1.0645	280,000	
55 minutes later	118	6.2	1.6400		
80 minutes later	120	6.4			

This lack of increase of haemoglobin and erythrocytes during physical exertion at the high altitude invalidates Willebrand's explanation of the concentration, which is also accepted by Zuntz and Schumburg. They believed that the blood was concentrated in exercise by the passage of fluid from the blood to the active muscles. If this explanation is correct the blood, during exertion, should have been concentrated as much on Pike's Peak as in Colorado Springs. These observations also show that loss of water from the body by evaporation from the lungs, and by increased sweating and urine formation is not an adequate explanation of the concentration that occurs at the low altitudes.

A series of ten abdominal pressure experiments was made in the same manner as at the lower altitude and in several the abdomen was also vigorously kneaded for some minutes before the weights were placed in position. The striking feature of these experiments was an opposite reaction from that obtained by a similar procedure at the low altitude, instead of an increase

in haemoglobin and red corpuscles a reduction in both of from 2 to 4 per cent was obtained. The following is a typical result from an experiment in which both kneading and pressure were employed:

	HAEMOGLOBIN	RED CORPUSCLES
Normal	123	6,744,000
Pressure 5 minutes	121	6,568,000
Pressure 13 minutes	120	6,496,000

These massage-pressure experiments show clearly why the blood failed to concentrate during exercise on Pike's Peak. The reserve of corpuscles had been depleted because of the great need of oxygen at the high altitude. At low altitudes the need for a greater supply of oxygen occurs only during muscular activity; at high altitudes the need is also felt during rest, with the result that even at the beginning of muscular work there are no corpuscles remaining in reserve, and therefore there is no increase with exercise.

The influence of adrenalin. Having found evidence that the splanchnic area is an important and very likely primary reservoir for the superabundant red corpuscles during inaction, the question arose as to how the extra supply was thrown into the general circulation during exercise. In view of the recent researches by Canon (7) and his pupils which show the relation of the adrenal medulla to muscular activity; and in view of the fact that v. Anrep (8) had shown that an increase of carbon dioxide in the blood, such as occurs in exercise, by stimulating certain nerve centers, caused the suprarenals to secrete more freely; and because it was well known that the circulation of the abdomen is shifted to the general circulation by this secretion, it occurred to us that we were dealing with another of the adaptive functions regulated by adrenalin. We, therefore, administered by mouth to a subject, when the stomach was empty, a Parke, Davis and Company preparation of adrenalin with the following results:

	HAEMOGLOBIN	ERYTHROCYTES	LEUCOCYTES
45 drops of adrenalin administered at 11.34 a.m.			
Normal	115	6,064,000	8,600
In 10 minutes	116	6,272,000	6,800
In 20 minutes	119	6,440,000	7,800
In 30 minutes	118	6,280,000	8,400
16 drops more administered 34 minutes later.			
40 minutes after first dose	121	6,472,000	
55 minutes after first dose	118	6,296,000	
70 minutes after first dose	117	6,176,000	

The first effect of adrenalin so administered would no doubt be a vaso-constriction of the splanchnic area and, as Edwards (9) has recently shown in a study of the compensatory distribution of the blood during stimulation of the splanchnic nerve, there would be an increased transfer of blood to the vascular circuits of the head and extremities sufficient to compensate for the diminished flow through the portal circuit. If then a large mass of red corpuscles has been side-tracked in the splanchnic area and blood plasma withdrawn, the constriction of the vessels of this large vascular area would force the stagnant mass of red corpuscles on toward the heart into the general circulation and thus give the increase in haemoglobin and red corpuscles found in the above record.

How account for the changes in the number of leucocytes and platelets? Several fragmentary observations show clearly that the changes occurring in the leucocytes and platelets as a result of physical exertion are not produced in the same manner as those in the haemoglobin, red corpuscles, and specific gravity. In three experiments on Pike's Peak the changes in the number of leucocytes and platelets were followed through a part of the process and they were found to react exactly as at the lower altitude. The white corpuscles increased as much as 57 per cent in a run, and the platelets decreased fully 28 per cent, while they later showed the customary overproduction. Thus we here find proof for the contention of other workers that the increase

in leucocytes in exercise can not be the result of the same reaction that increases the number of red corpuscles. At high altitudes exercise squeezes the lymph out of the muscles and drives it toward the heart just as at low altitudes, and with it would be carried the leucocytes gathered from the tissues. The supposition that the leucocytes are derived from the tissues other than the splanchnic area was further supported by the abdominal pressure determinations made in Colorado Springs, some of which are recorded in table 2. In these the leucocytes were only increased from 4 to 7 per cent, while in exercise they increased 14 to 130 per cent.

Some connection appears to exist between the mononuclear or lymphocyte index and the variation in the number of platelets. For some minutes after the exertion there appears to be a destruction of platelets which in turn very likely leads to a disappearance of the lymphocyte elements. Brown (10) has shown when an excessive demand for platelets exists that the mononuclear and transitional cells in the marrow, spleen, and blood may form platelets. Here then is a possible explanation for the decrease in lymphocytes.

The picture of the blood changes in exercise may be briefly summarized. During muscular inaction a large mass of the blood is diverted to the splanchnic area, where it probably stagnates and gives up plasma as lymph. There is also throughout the remainder of the body, especially in the limbs, an accumulation of lymph. With the onset of muscular activity the carbon dioxide content of the blood rises, this carbon dioxide stimulates the central nervous centers which regulate the secretion of the suprarenal glands, hence the output of adrenalin is increased. The adrenalin causes a constriction of the blood vessels of the splanchnic area, this forces the stagnant red corpuscles into the general circulation, thus giving the rise in specific gravity, haemoglobin, erythrocyte, and leucocyte content of the peripheral blood. Further the contraction of the voluntary muscles accelerates the flow of lymph, throwing lymph rich in leucocytes into the blood. The increase in red corpuscles and haemoglobin makes it possible to supply more readily the greater

demand for oxygen made by the active muscles. Shortly after the close of the exercise the carbon dioxide content of the blood falls below normal, as a result the discharge of adrenalin becomes subnormal and the blood once more accumulates in the splanchnic area, so that there is a gradual return to the normal composition and even a temporary subnormal content in red corpuscles.

BLOOD CHANGES DURING A PERIOD OF ATHLETIC TRAINING

Some data has been accumulated to show the influence of training upon the blood. This phase of the work was disappointing since we were unable to secure the hearty coöperation of the subjects that was necessary to furnish a complete picture of changes. We had only three men who were available both before they began training and during the season of spring athletics. One subject who trained for the two mile run was followed closely, the others gave opportunity for only a few observations during each period. Briefly, the results of these studies show an increase in haemoglobin and red corpuscles during the period of training for two of the subjects and no change in the third, For the subject followed closely the average content of haemoglobin in the foreperiod was 111.9 and during the height of training 115.8, which is an increase of 3.6 per cent. The red corpuscles averaged in the foreperiod 6.2 millions and 6.5 millions per cubic millimetre while in training, or an increase of 4.8 per cent. The second subject showed an average increase of 9.4 per cent in haemoglobin, this rose, as a result of training, from an average of 117 to 128. The erythrocytes increased from 6.1 to 7.2 millions or 18 per cent. No change was found to occur in the number of leucocytes and platelets. For the subject followed most carefully differential counts of the leucocytes were made in sufficient numbers in both periods to permit a comparison. There was an increase in the lymphocyte index of 4.1 per cent. In the untrained condition the lymphocyte index averaged 37, while during the period of splendid condition it was 38.75.

A series of determinations of the total oxygen capacity of the blood and total blood volume was made on the one subject closely studied. The results of this study appear in Table 3. The determinations were made by the carbon monoxide method of Haldane and Lorrain Smith (11).

No change in total oxygen capacity and blood volume is shown by these determinations. With the haemoglobin of this subject's blood only increased 3.6 per cent, it is unlikely that the method of determining total oxygen capacity is exact enough to detect so small a change.

TABLE 3 (SUBJECT I)

DATE	PERCENTAGE OF HAEMOGLOBIN	PERCENTAGE OXYGEN CAPACITY	TOTAL OXYGEN CAPACITY	TOTAL VOLUME OF BLOOD
			cc.	*cc.*
February 13	113	20.9	1032	4938
February 20	109	20.2	1039	5144
February 27	109	20.2	1003	4965
March 24[1]	113	20.9	1037	4962
April 17	112	20.7	1045	5048
May 3	116	21.5	1057	4916

[1] Training began March 1.

SUMMARY

1. The immediate influence of physical exertion upon the blood of the peripheral capillaries was one of concentration in which the percentage of increase varied as follows: haemoglobin 3.5 to 10.9, erythrocytes 3.2 to 22.8, and leucocytes 13.8 to 130.2. The specific gravity increased proportionately with the red corpuscles.

2. Within a few minutes after the close of the exertion the blood began to be diluted and this usually resulted in a subnormal specific gravity and content of haemoglobin and red corpuscles.

3. At the end of the exertion the differential counts of the leucocytes showed no change, but very soon the polymorphonuclears increased 9 to 45 per cent and the total number of mononuclear elements decreased 14 to 55 per cent.

4. The number of platelets was unchanged at the close of the exercise. However, there then followed a period of decrease which reduced them 17 to 30 per cent. Later there was a period of overproduction which carried the number above the normal 17 to 25 per cent.

5. Long continued and severe exertion apparently does not cause an increased destruction of red corpuscles.

6. A repetition of a run when the blood has passed into the subnormal condition practically restores the maximum concentration.

7. Abdominal massage and pressure raise the content of haemoglobin and red corpuscles in the blood of the peripheral capillaries.

8. A tightly drawn belt or pressure on the abdomen following exertion prevents in large measure the dilution of the blood.

9. At an altitude of 14,109 feet the blood does not concentrate during exercise. However, the changes in white corpuscles and platelets occur as at the lower altitude.

10. Abdominal massage and compression cause a dilution of the blood at the high altitude.

11. Adrenalin administered by mouth causes the blood in the peripheral capillaries to be concentrated.

12. During a period of training for spring athletics the percentage of haemoglobin and the number of red corpuscles were found to increase. The number of leucocytes and platelets was not changed. The mononuclear elements were increased 4.1 per cent. No change in total oxygen capacity or total blood volume was found.

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THE NEUTRALIZING POWER OF SALIVA IN ITS RELATION TO DENTAL CARIES

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A cursory examination of the dental literature of the last few years shows one topic—the influence of the salivary secretion upon dental caries—to be of vital interest, a topic often discussed and about which many articles have been written.

The importance of the saliva in the etiology of dental caries is a well established fact, but the variability in the composition of the secretion has rendered the exact study of the saliva difficult.

The history of the work done and the theories advanced, controverted, and again advanced, is voluminous. The whole subject, in fact, has been so well discussed by others that a repetition here or references to all authors would involve a resumé which seems at this time unwarranted. The omission of many names then, is not prompted by lack of courtesy, but from the desire to omit all possible extraneous material and only those authors are mentioned whose work has a direct bearing on the problem in hand.

The different phases of the subject which have been under discussion at various times are numerous. In Johnson's (2) Operative Dentistry is found an elaborate theory by G. W. Cook in which he undertakes to prove that mucic acid may be found in the mouth and that it is responsible for many eroded areas on the tooth structure. His suggestion, however, is insufficiently supported by scientific evidence. According to our present day knowledge of physiology and pathology, mucic acid never occurs in the human body. Rose (14) has shown that mucic

acid is not an intermediary product in the metabolism of galactose-yielding sugars. Furthermore Thierfelder (15) working on the isomer of mucic acid, saccharic acid, says that although this acid results from the oxidation of glycuronic acid in vitro there is no evidence that this change can take place in the animal organism.

Pickerill (3) has attacked the problem from the standpoint of reaction to different stimuli and has shown in tabular form the increase in alkalinity of a saliva which has been activated by acid substances.

Important contributions have been made by Gies (4) and his co-workers. In a series of carefully conducted experiments covering over three years they have given us results which have thrown much needed light on the problem in hand.

He fails to find a definite relation between the general composition of the saliva and the condition of the teeth and notes that the systemic condition of the patient has a direct bearing upon susceptibility to dental caries. Bunting (5), Bödecker (6), Howe (8) and others have given us the benefit of their theories but unfortunately they fail in nearly every instance to describe their experiments and their theories can hardly be regarded seriously without such detail.

Kirk (7) brings out some important data in relation to indicators in the titration of saliva, by comparing results obtained with methyl orange, congo-red, litmus, phenolphthalein and thymolphthalein. He defines Pickerill's *index of alkalinity* as an *index of the power of the mucin to clear the saliva of its acid content* by forming an acid mucin coagulum. In fact he seriously questions the reliability of Pickerill's results pointing out quite definitely that the use of methyl orange in weak solution depends too much upon the personal equation to insure the obtaining of trustworthy data.

The reaction of saliva is amphoteric, that is to say it exhibits the power of neutralizing both acids and bases. Many of the body fluids show this phenomenon. Fresh milk is amphoteric; urine is often amphoteric to litmus, i.e., it will show both an acid and alkaline reaction to that indicator. Sutton (11) says "Saliva

which is generally neutral to litmus is always strongly alkaline to lacmoid or congo-red and acid to tumeric." The indicator-method of neutrality determination does not show the exact neutrality point. The method is comparable with itself and not with the electrolytic method of determining neutrality.

The alkalinity of the saliva has been variously stated as being due to dibasic sodium phosphate and also sodium carbonate. Raymond (12) quotes Chittenden and Richards (13) as saying that "*Human saliva contains normally no sodium carbonate whatever* the alkalinity, as indicated by litmus, lacmoid, et cetera, is due to hydrogen alkali phosphates (and is equivalent to 0.08 to 0.10 per cent Na_2CO_3) and to possibly some alkali bicarbonate. Mixed saliva invariably reacts acid to phenolphthalein." Taylor (16), on the other hand, claims that "Fresh saliva is quite neutral but becomes faintly alkaline as the CO_2 is dissipated, later it becomes acid again through bacterial action." Allaria (18) states that his experiments "Prove the saliva from breast-fed babies to be acid." Gies (4) reports at the inception of his work that "In practically every instance the reaction was alkaline to both litmus and lacmoid and in every case was acid to phenolphthalein." All of Gies' reports express acidity.

There seems to be a little confusion among the different experimenters in regard to the results obtained. This may be due to a lack of observance of standard conditions in obtaining the samples or to different indicators used.

The choice of indicators in this work is not large. The dissolved gases, as CO_2, precludes those indicators which are affected by that substance. Boiling the sample to rid it of this gas is unwarranted for the proteids are thereby coagulated. The indicators chosen should possess the greatest sensibility, should show a definite color change, and should change at the point nearest the hydrogen ion concentration of water, viz. H^{10-7}. Phenolphthalein possesses these requirements changing between H^{10-8} and H^{10-9}; to it saliva reacts acid with the exception of a few instances to be mentioned later. Para-nitro-phenol changing between H^{10-6} and H^{10-7} was chosen as the second indicator and, although the color change is not so satisfactory as that of phenol-

phthalein, still, when the ammonium salt is formed in titration, the yellow color shows very definitely against the turbid saliva. Methyl red was used at first but was discarded later in preference to para-nitro-phenol. Methyl orange was not used on account of the indefinite color change in solutions weaker than $\frac{N}{10}$. Both Sutton (11) and Treadwell-Hall (22) question the accuracy of methyl orange in weak solutions.

The following solutions were prepared: HCl $\frac{N}{10}$, NaOH $\frac{N}{10}$, NH_4OH $\frac{N}{10}$. These were carefully standardized and the standardization confirmed every two weeks. From these tenth normal solutions two hundredth normal were made by diluting 50 cc. of $\frac{N}{10}$ to 1 litre. This strength of solution was used throughout the work. These also were standardized every two weeks against the $\frac{N}{10}$ solutions.

All reports are based on 10 cc. of saliva as samples and titration figures indicate the number of cubic centimeters of $\frac{N}{200}$ HCl and $\frac{N}{200}$ NaOH. Temperature conversion factors were computed for every 4°C. but as the temperature variation was within 10°C. during the entire experiment it was found unnecessary to correct for this variation in the use of $\frac{N}{200}$ solutions.

The normal resting saliva is a relative term. We have no means of knowing what is normal or what is resting. Conditions in the mouth vary not only from day to day but probably from hour to hour. Whether this is due to local conditions in the mouth entirely is an open question. The psychic influences are constantly changing and how much this affects the secretion of the saliva we may judge from the classical experiments performed by Pavlov (9) and others. Pavlov has shown that even the rattling of a cart in the street below will excite the salivary secretion of a dog. Heidenhain also has shown that by the use of weak induction shocks to the *chorda tympani* nerve he could cause the submaxillary glands to secrete a thin copious saliva. By stimulation of the *sympathetic* the flow was changed and became thick and turbid. These experiments explain to a certain extent the difficulties encountered in obtaining a "normal resting saliva," and the consequent variation in analyses of saliva from the same individual. The determination of a constant

normal resting saliva involves, at present too many x's in the equation and has not been made, so far as the writer is aware.

The samples of saliva for this research work were obtained largely through the kindness of the authorities of various hospitals, asylums and penal institutions. Certain inmates gladly cooperated, after explanations, in furthering the work of the writer. In all 104 cases were examined, but voluntary coöperation was obtained in only 96 cases.

Following the suggestion of Dr. Gies (4) the same type of bottle was used in the collection of every sample—a 2 oz. glass stoppered "salt mouthed" bottle. The subject, (or more correctly, the person), was requested to expectorate in the bottle without making a conscious effort to produce the saliva. The bottle was kept tightly closed during the intervals which elapsed between the actual acts of expectorating. Before the sample was collected the condition of the mouth was noted, also any habits in regard to tobacco, alcohol or diet. Also the following points were observed:

1. The samples to be comparative throughout were obtained by natural methods or by the ordinary methods of stimulation; or, if in the resting stage, under known, or at least as constant conditions, as possible.

2. The patient should be as nearly normal as possible in salivary activity.

The data obtained from the several hospitals were compiled only from those convalescent patients who were not taking any medication (except as noted). The data from the prison inmates were compiled from individuals who were in good health and who were doing the regular prison routine work. The data from the inmates of the insane asylums were taken from those who were not entirely mentally deficient for the obvious reason that it was only that class which could be made to understand what was wanted. The diets which were carefully studied and compared throw little light upon the results from the standpoint of Pickerill's work, for, in one instance, where the carbohydrate content is in excess one would almost expect, judging from the results of Pickerill (3), to find a higher acidity than is reported.

METHODS OF ANALYSIS

For Alkalinity. Ten grams of the well mixed sample are placed in a casserole diluted with 20 cc. of distilled water at 20°C. and free from CO_2. About eight drops of an aqueous solution of para-nitro-phenol are added and $\frac{N}{200}$ HCl run in, in slight excess. The mucin is thereby precipitated and the determination of the end point made easier in the clarified solution. Next $\frac{N}{200}$ NH_4OH is added until the first faint trace of a yellow color is discernible. The difference between the amount of acid added in the first instance and the amount of alkali added in the second, equals the alkalinity in terms of cubic centimeters of $\frac{N}{200}$ HCl and is thus reported.

For Acidity. A second 10-gram sample is diluted as above and about four drops of phenolphthalein are added. Then $\frac{N}{200}$ NaOH is run in until the first permanent pink color appears. The cubic centimeters of alkali added equals the acidity of the sample in terms of cubic centimeters of $\frac{N}{200}$ NaOH and are thus reported.

The double titration in the determination of alkalinity while not absolutely necessary, is advisable as it makes for accuracy. For instance the HCl may be added to a point of decoloration of the indicator but this exact point is obscured in many instances by the color of the sample itself. With the precipitation of the mucin, by the addition of the excess of acid, this difficulty is overcome.

Since phenolphthalein does not react satisfactorily with ammonia it was necessary to employ NaOH in the determination of acidity, while the NaOH used in conjunction with the para-nitro-phenol was not so satisfactory as NH_4OH for the reason that the latter gives a more definite end point with this indicator.

In the analyses of samples from those institutions outside of San Francisco the apparatus and solutions were taken and analyses made at the respective places at the time of taking the sample. This insured a direct comparison of all analyses presented, since only one of the samples was allowed to stand for longer than an hour before titrating.

The following were the results obtained (tables 1–8). In the tables the word "immunity" is used in the sense of freedom from caries. Those cases which on careful examination showed no fillings and no areas of decay have been referred to as "absolute immunity." Those cases which showed fillings but not areas of decay are referred to as "present immunity." The

TABLE I

Present immunity, with care

NUMBER OF PATIENT	cc. $\frac{N}{200}$ HCl	cc. $\frac{N}{200}$ NaOH	NUMBER OF PATIENT	cc. $\frac{N}{200}$ HCl	cc. $\frac{N}{200}$ NaOH
1	23.00	4.50	3	9.94	4.82
	25.50	4.30		8.94	5.02
	28.40	4.90		9.20	5.30
	16.02	5.86		18.10	3.40
	16.80	6.34			
	12.06	5.72	11	10.70	5.80
	13.70	5.42		11.70	5.45
	20.20	6.42			
			14	7.58	18.30
2	11.00	12.60			
	13.70	14.60	36	21.64	4.41
	14.06	13.10	40	9.90	8.00
	11.02	10.50	41	17.04	11.22
	7.36	12.34	55	9.04	4.02
	9.40	9.46	61	9.50	4.30
	10.72	9.34	65	10.60	9.86
	8.32	12.10	67	13.70	4.97
	12.90	8.20	70	17.90	7.78
				14.45	5.10
3	17.70	4.50	74	19.80	8.00
	19.50	5.60	75	26.60	4.30
	17.50	5.40	89	24.25	2.70
	12.50	5.00	103	7.80	7.53

term "with care," denotes that there is evidence of the patient using the toothbrush, i.e., "caries with care," denotes that a carious condition exists at the time of taking the sample, and that the patient cleanses the mouth and endeavors to keep the teeth as free from food débris as possible.

In table 1 is presented the results of the titration of "normal resting saliva" collected from the mouths of those patients who habitually take good care of their teeth and who at present show

no signs of caries. In the case of the first three patients the tests extended over a period of days. The samples were taken at the same time each day and titrated at once. The wide variation shown not only between the several patients but also daily by the same patient argues against the theory that acidity of the *resting saliva*, (that is of the saliva which is contaminated with the microorganisms of the mouth and food débris,) is an influencing factor in the *causation* or *progress* of dental caries, and also against the view that the alkalinity of resting saliva acts as a protecting

TABLE II

Present immunity, without care

NUMBER OF PATIENT	cc. $\frac{N}{200}$ HCl	cc. $\frac{N}{200}$ NaOH	NUMBER OF PATIENT	cc. $\frac{N}{200}$ HCl	cc. $\frac{N}{200}$ NaOH
5	24.60	5.60	50	7.14	7.52
	24.70	7.20	51	11.32	5.54
	22.20	9.80	53	1.82	12.02
	19.30	14.40	54	12.52	5.80
			58	21.10	alkaline (trace)
8	10.30	9.26	59	11.86	6.00
	14.08	11.86	64	11.40	8.67
	14.20	4.50	72	29.10	3.44
	22.22	7.60		22.50	6.28
				29.10	3.44
32	8.96	7.20	73	9.75	15.60
43	15.14	4.80	79	16.50	2.13
46	13.38	5.10	83	21.55	7.00
48	17.26	2.98	96	28.80	6.97
49	2.66	7.62	97	25.60	4.45

agent against caries. It will be shown later, however, that the *activated saliva* produced under definite stimulation, *is* an influencing factor in the production and maintenance of immunity.

For instance:

In patient No. 2 Table 1 the variation is 6.70 cc. HCl; 6.40 cc. NaOH.
In patient No. 3 Table 1 the variation is 9.56 cc. HCl; 1.20 cc. NaOH.
In patient No. 5 Table 2 the variation is 5.40 cc. HCl; 9.80 cc. NaOH.
In patient No. 8 Table 2 the variation is 11.92 cc. HCl; 7.06 cc. NaOH.
In patient No. 4 Table 3 the variation is 8.58 cc. HCl; 3.57 cc. NaOH.
In patient No. 6 Table 4 the variation is 5.60 cc. HCl; 1.30 cc. NaOH.
In patient No. 20 Table 4 the variation is 16.20 cc. HCl; 8.02 cc. NaOH.

TABLE III

Carious, with care

NUMBER OF PATIENT	cc. $\frac{N}{200}$ HCl	cc. $\frac{N}{200}$ NaOH	NUMBER OF PATIENT	cc. $\frac{N}{200}$ HCl	cc. $\frac{N}{200}$ NaOH
4	18.40	4.80	21	15.36	2.74
	19.50	4.40	37	13.88	5.06
	17.30	3.65	45	7.20	7.42
	19.70	3.30	60	12.40	4.60
	11.44	5.50	71	2.95	1.70
	11.22	6.80	80	17.75	7.70
	13.00	5.58	81	28.80	5.82
	20.80	3.23	101	14.20	7.67
16	14.60	8.30	102	15.70	7.40
	14.00	12.90	104	17.30	17.40

TABLE IV

Carious, without care

NUMBER OF PATIENT	cc. $\frac{N}{200}$ HCl	cc. $\frac{N}{200}$ NaOH	NUMBER OF PATIENT	cc. $\frac{N}{200}$ HCl	cc. $\frac{N}{200}$ NaOH
6	16.60	7.10	39	7.20	9.22
	13.30	8.40	42	12.30	9.90
	11.00	7.50	47	8.16	3.10
9	4.70	9.70	52	16.32	1.00
	3.30	12.00	56	16.64	2.64
10	9.80	29.30	57	15.46	1.76
	4.20	23.30	62	7.90	12.70
15	13.38	3.12	69	11.50	4.53
	14.00	2.90	76	18.50	7.00
	16.50	2.20	77	17.45	15.00
17	9.40	8.10	82	8.95	18.33
18	2.80	22.10	84	28.90	3.70
	11.70	15.00	85	17.40	22.00
20	4.70	13.20	86	28.90	27.00
	12.90	5.18	87	24.20	2.76
	19.20	7.20	88	16.65	7.90
	20.90	5.00	90	19.95	10.00
	16.60	5.43	91	21.80	9.40
22	8.15	6.20	93	24.00	11.70
23	6.90	8.94	94	24.45	11.10
26	12.52	3.16	95	29.35	5.00
33	10.60	5.00	98	14.00	9.70
34	10.60	3.80	99	36.60	22.94
35	14.40	10.08	100	25.35	18.30
38	12.60	3.42			

From the above it is readily seen that no "normal" resting saliva exists for a variation of 16.20 cc., is rather too great to admit of establishing a factor which will definitely represent the "normal" condition of the saliva.

On further comparison also of tables 1 and 2, one observes that the looked for difference in clean or unclean mouths does not exist. That is, the acidities in resting saliva are relatively no higher in the one case than in the other. In table 2, patient 58, a notation in reference to the reaction to phenolphthalein is made. This man although an inmate of the institution for nearly four years is still unclassified. His physical condition is good and he is accorded the freedom of those who work outside. The writer can advance no theory for this exception in reaction.

TABLE V

Pyorrhea, with care

NUMBER OF PATIENT	cc. $\frac{N}{200}$ HCl	cc. $\frac{N}{200}$ NaOH
24	7.16	1.18
27	6.00	8.10

In noting the differences in acidity and alkalinity in tables 1, 2, 3 and 4, we fail to find any constant factor on which to base the assumption that the resting saliva in an unclean mouth has a higher acid or alkali content than the saliva from a clean mouth. In fact the condition of the saliva as resting, is too variable to establish a relationship between the acidity and alkalinity of a clean or unclean mouth, and the acidity and alkalinity of a carious or an immune mouth.

In tables 5 and 6 the number of cases presented is too small to attempt even an inference as to the relationship or lack of it that exists between pyorrhea and the reaction of saliva.

Tables 7 and 8 likewise show a lack of relationship. The high acidity as noted in table 7, patient 66, is noteworthy. In this particular case the form of the teeth invited decay. The embrasures between were not particularly well marked and the

TABLE VI

Pyorrhea, without care

NUMBER OF PATIENT	cc. $\frac{N}{200}$ HCl	cc. $\frac{N}{200}$ NaOH	NUMBER OF PATIENT	cc. $\frac{N}{200}$ HCl	cc. $\frac{N}{200}$ NaOH
31	9.84	6.40	83	21.55	7.00
43	15.14	4.80	84	28.90	3.70
62	7.90	12.70	85	17.40	22.00
82	8.95	18.33	86	28.90	27.00
73	9.75	15.60	87	24.20	2.76
72	29.10	3.44	90	19.95	10.00
	22.50	6.48	91	21.80	9.40

proximal contacts rather broad. The teeth were not of the bell crowned type but rather straight and flat.

All of the foregoing tables and above remarks have been in more or less opposition to the views held by the majority of the dental profession and may be said to be negative in character.

The following tables are, however, constructive in character, rather than destructive, and point to a physiological condition constantly present but which requires further study and experimentation.

It occurred to the writer that it might be interesting to note the stimulation reaction of saliva (using the same stimulus) in the mouths of those who have carious teeth and those who are not susceptible to caries. Chittenden and Richards (13) noted in 1898 "That mechanical stimulation, as chewing a tasteless substance, and alcohol, ether, gin, whisky taken into the mouth all lead to the outpouring of a saliva richer in alkaline reacting salts and amylolytic power, than the secretion coming *without* stimulation."

TABLE VII

Absolute immunity, with care

NUMBER OF PATIENT	cc. $\frac{N}{200}$ HCl	cc. $\frac{N}{200}$ NaOH
29	16.26	3.48
30	13.20	4.20
66	14.60	17.10

TABLE VIII

Absolute immunity, without care

NUMBER OF PATIENT	cc. $\frac{N}{200}$ HCl	cc. $\frac{N}{200}$ NaOH
25	10.38	7.20
28	15.40	3.32
44	17.00	2.80

It was along this line that the writer started the second series of experiments. The samples for these experiments were obtained as follows. A "normal resting saliva" was collected in the usual way. Then a small piece of white paraffine was given the individual with the instruction not to chew until the wax had become softened. When soft the chewing was commenced and continued until about 20 cc. of sample had been obtained. The time necessary for this was usually from fifteen to twenty minutes. The results are noted in tables 9 to 13 inclusive. In table 9 one notes in comparing the figures in column 3 with those in column 6 an astonishing drop in acidity, viz., from 17.10 cc. to 0.80 cc. and in comparing columns 2 and 5 a rise in alkalinity from 14.60 cc. to 43.02 cc. This interesting relation between

TABLE IX

Paraffine stimulus. Absolute immunity, with care

NUMBER OF PATIENT	NORMAL RESTING SALIVA			ACTIVATED SALIVA			
	cc. $\frac{N}{200}$ HCl	cc. $\frac{N}{200}$ NaOH	Neutralizing power	cc. $\frac{N}{200}$ HCl	cc. $\frac{N}{200}$ NaOH	Neutralizing power	Salivary factor
66	14.60	17.10	31.70	43.02	0.80	43.82	72.3

the drop of the acidity on the one hand and the rise of alkalinity on the other can best be shown in the following way.

In considering the reaction of the blood and some of the other tissue fluids Henderson (19), Robertson (20), and others, have shown that in measuring the power of the blood to maintain neutrality, this power may be estimated by *adding* the *acid* and *alkali titrations*; thus measuring the amount of reagent necessary to change the reaction of the blood from a definite alkalinity to a definite acidity.

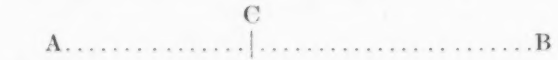
In discussing this subject with Dr. Robertson he expressed the matter in the following words:

> In seeking for a method of measuring the power of saliva to maintain itself neutral it is necessary to bear in mind the fact that no fluid can remain utterly unchanged in reaction as a result of adding acids or alkalies to it. Some change will ensue as a result of these additions

and that fluid possesses the greatest power of maintaining itself neutral or nearly neutral in which a given addition of acid or alkali produces the least change, or, conversely, in which a *given* change in acidity is brought about by the addition of the largest amount of reagent. We may measure the power of saliva to maintain its neutrality, therefore, in either of two ways—we may measure the amount of change produced by adding a given proportion of acid or alkali to the saliva—(the smaller the amount of change the greater the power of the saliva to maintain itself neutral) or, which is more convenient, we may measure the quantity of reagent required to change the saliva from one definite reaction to another definite reaction. The greater this quantity, the greater the power of the saliva to maintain itself neutral.

The reaction of normal resting saliva corresponds to no definite acidity or alkalinity—it varies not only from individual to individual but doubtless from moment to moment in the same individual, since the saliva is momentarily exposed to countless variable influences which must and do affect its precise reaction.

In order, therefore to measure the quantity of acid (or alkali) required to change the saliva from one given reaction to another given reaction we must first reduce all the samples of saliva to one definite condition (reaction) and, starting from this definite reaction estimate how much acid (or, reversely, alkali) must be added to this saliva to change it to another definite condition (reaction).



Let "A B" be any quantity, for instance a distance or a volume (e.g., cubic centimeters of standard acid solution) which we desire to measure. If it be convenient, it is obvious that we can just as well measure "A B" by adding the distance "A C" to the distance "C B" as by measuring "A B" in one operation.

Applying this principle to the measurement of the power of saliva to maintain itself neutral, the position of "C" will represent the variable, fluctuating reaction of normal resting saliva. "A" will be a definite reaction on the acid side of neutrality (i.e., neutrality to para-nitrophenol) and "B" a definite reaction on the alkaline side of neutrality (i.e., neutrality to phenolphthalein). The distance "A B" is the number of cubic centimeters of acid required to change the saliva from reaction "B" to reaction "A" or, reversely, the number of cubic centimeters of alkali required to change the saliva from reaction "A" to reaction "B."

This distance is the sum of the distances "A C" and "C B," this is, of the number of cubic centimeters of acid required to bring the given sample of saliva to neutrality to para-nitro-phenol and the number of cubic centimeters of alkali which is required to bring the sample to neutrality to phenolphthalein. In other words *by adding together the results of the above titrations with acid and alkali we obtain a true measure of the power of the given samples of saliva to maintain their neutrality.* Neither of the titration values taken apart, affords such a measure, for, as explained above, the reaction of resting saliva (position of C in the above diagram) varies from moment to moment in consequence of events which we cannot estimate or define, hence the separate titres (distances

TABLE X

Paraffine stimulus. Present immunity, with care

NUMBER OF PATIENT	NORMAL RESTING SALIVA			ACTIVATED SALIVA			
	cc. $\frac{N}{200}$ HCl	cc. $\frac{N}{200}$ NaOH	Neutralizing power	cc. $\frac{N}{200}$ HCl	cc. $\frac{N}{200}$ NaOH	Neutralizing power	Salivary factor
1	20.20	6.42	26.62	37.82	2.10	39.92	66.00
2	12.90	8.20	21.10	47.50	1.70	49.20	43.00
3	14.70	3.40	18.10	37.40	1.70	39.10	46.00
67	13.70	4.97	18.67	34.95	1.13	36.08	51.70
70	17.90	7.78	25.68	49.45	2.50	51.95	49.40
70	14.45	5.10	19.55	43.80	1.20	45.00	43.60
74	19.80	8.00	27.80	45.35	1.90	47.25	58.80
75	26.60	4.30	30.90	50.36	1.93	52.29	59.10
89	24.25	2.70	26.95	32.80	1.40	34.20	75.90
103	7.80	7.53	15.33	14.65	3.90	18.55	82.65

A C or C B) varying from moment to moment in saliva of every individual *but their sum, "A B," remains, it is probable, relatively constant and characteristic.*

The samples for the following tables are from two classes of individuals. First, those persons who were regularly engaged in their daily routine as students, members of the office force, etc. Second, those persons, convalescent in the surgical wards of the different hospitals, who were taking no medication (except as noted) and who were on full diet.

Referring again to table 9 it is observed in this case of absolute immunity that the ratio, expressed in percentage, between the

neutralizing power of normal resting saliva and that of the activated saliva which the writer terms the "salivary factor," is 72.3.

Table 10 which is composed of cases of present immunity with care shows a relationship similar to the preceding table. The "salivary factor" with one exception is below 80, fluctuating between 43 and 75.9. Concerning the exception, patient Number 103, the writer will not attempt to theorize. The clinical record shows, however, an acid urine.

In table 11 the variation is nearly the same as in the two preceding tables and *all* percentages fall below 80. In patient

TABLE XI

Paraffine stimulus. Present immunity, without care

NUMBER OF PATIENT	NORMAL RESTING SALIVA			ACTIVATED SALIVA			
	cc. $\frac{N}{200}$ HCl	cc. $\frac{N}{200}$ NaOH	Neutralizing power	cc. $\frac{N}{200}$ HCl	cc. $\frac{N}{200}$ NaOH	Neutralizing power	Salivary factor
8	22.22	7.60	29.82	57.55	0.90	58.45	51.00
64	11.40	8.67	20.07	30.90	6.00	36.90	53.85
65	10.60	9.86	20.46	20.00	6.00	26.00	78.70
72	29.10	3.44	32.54	43.22	3.20	46.42	70.10
72	22.50	6.28	28.78	36.80	2.70	39.50	72.90
73	9.75	15.60	25.35	25.90	5.97	31.87	79.50
79	16.50	2.13	18.63	35.14	—0.40	35.14	53.00
83	21.55	7.00	28.55	39.20	4.00	43.20	66.10
96	28.80	6.97	35.77	56.20	0.70	56.90	62.90
97	25.60	4.45	30.05	37.70	1.40	39.10	76.90

79 the activated saliva was alkaline to phenolphthalein 0.40 cc. $\frac{N}{200}$ HCl. This was one of the very few exceptions to the general rule that saliva reacts acid to that indicator. Duplicate determinations were made on successive days the alkaline titration figure being 0.30 cc. $\frac{N}{200}$ HCl in one instance, and 0.70 cc. $\frac{N}{200}$ HCl in another. The salivary factor is constant within certain limits for the same individual when the same conditions obtain at the time each sample is taken. For instance, patient 72, varied on successive days between 70.1 and 72.9 per cent.

Turning now to table 12, "carious with care," a marked difference in the salivary factor is at once apparent; for in all

these cases it is above 80, fluctuating between 84 and 119.3. In this, and the following table, all degrees of carious conditions are presented.

Table 13, "carious without care," shows two exceptions. In the first case, Number 20, the patient was suffering from acute nephritis, hip arthritis, tuberculosis, and syphilis. These facts may, or may not, explain the low factor. In the second case, Number 94, the patient was taking sodium phosphate. Patient 93 is an example of an abnormally high salivary factor. The individual was suffering from peripheral neuritis and the disordered reflexes as evidenced in other ways might account

TABLE XII

Paraffine stimulus. Carious with care

NUMBER OF PATIENT	NORMAL RESTING SALIVA			ACTIVATED SALIVA			
	cc. $\frac{N}{200}$ HCl	cc. $\frac{N}{200}$ NaOH	Neutralizing power	cc. $\frac{N}{200}$ HCl	cc. $\frac{N}{200}$ NaOH	Neutralizing power	Salivary factor
4	20.80	3.23	24.03	26.40	2.00	28.40	84.6
80	17.75	7.70	25.45	24.40	2.52	26.92	94.5
81	28.80	5.82	34.62	32.74	2.22	34.96	99.0
101	14.20	7.67	21.87	15.05	5.50	20.55	106.4
102	15.70	7.40	23.10	19.45	4.00	23.45	98.5
104	17.30	17.40	24.70	25.80	3.30	29.10	119.3

for the high factor. In another case, patient 77, two sets of analyses are shown. The first set are from samples taken between 9.30 and 10.30 a.m. and the second from samples taken at 6 a.m. immediately on waking. The latter sample was not titrated until three hours after it had been collected and through error had been kept in a warm room in the interim. The writer suggests that these conditions might account for the difference in the two salivary factors for the same individual.

The following cases were all suffering from rampant caries:

Table 12, Patient 104.
Table 13, Patient 86.
Table 13, Patient 90.
Table 13, Patient 100.

It is interesting to note that in all these examples the salivary factor is abnormally high, being 119.3, 121.8, 132.8, 122.9, respectively. The number of cases, however, is so small that it would be presumptuous to infer that rampant caries is always associated with an exceptionally high salivary factor. It would appear, however, legitimate to conclude that in those persons susceptible to caries the ratio of the neutralizing power of the

TABLE XIII

Paraffine stimulus. Carious without care

NUMBER OF PATIENT	NORMAL RESTING SALIVA			ACTIVATED SALIVA			
	cc. $\frac{N}{200}$ HCl	cc. $\frac{N}{200}$ NaOH	Neutralizing power	cc. $\frac{N}{200}$ HCl	cc. $\frac{N}{200}$ NaOH	Neutralizing power	Salivary factor
20	20.90	5.00	25.90	39.80	3.00	42.80	60.5
76	18.50	7.00	25.50	22.80	2.13	24.93	102.0
77	17.45	15.00	32.45	26.40	4.98	31.38	103.4
77 (6 a.m.)	24.75	22.00	46.75	32.92	6.00	38.92	120.0
82	8.95	18.33	27.28	24.35	4.30	28.65	95.0
84	28.90	3.70	32.60	30.50	0.50	31.00	105.0
85	17.40	22.00	39.40	31.95	4.63	36.58	107.7
86	28.90	27.00	55.90	40.50	6.40	46.90	121.8
87	24.20	2.76	26.96	26.75	2.00	28.75	93.8
90	19.95	10.00	29.95	20.15	2.47	22.62	132.8
91	21.80	9.40	31.20	30.50	3.70	34.20	91.2
93	24.00	11.70	35.70	19.50	5.73	25.23	160.6
94	24.45	11.10	35.55	41.00	9.40	50.40	70.5
95	29.35	5.00	34.35	30.75	4.00	34.75	98.9
98	14.00	9.70	23.70	22.86	2.00	24.86	95.3
99	36.60	22.94	59.54	45.40	12.60	58.00	102.6
100	25.35	18.30	43.85	32.55	2.97	35.52	122.9

normal resting saliva compared to that of the activated saliva differs from the ratio observed in those immune from caries.

Assume for example that the neutralizing power of the normal resting saliva is represented by 25 cc. and that of the activated saliva by 25 cc.; the ratio between the two is $\frac{25}{25}$ or unity or 100 per cent. Further, if the neutralizing power of the normal resting saliva is 20 and that of the activated saliva is 15 the ratio is $\frac{20}{15}$ or 133 per cent. In this latter case the normal resting saliva is the more potent of the two secretions in maintaining the neutrality of the oral cavity.

These two examples illustrate the ratio obtained in carious conditions. Consider now, the immune ratio. Assume that 15 cc. equals the neutralizing power of the normal resting saliva and that 25 cc. equals that of the activated saliva, then $\frac{15}{25}$ equals 60 per cent. In other words the power of the normal resting saliva is only 60 per cent of the power of the activated saliva and a reserve neutralizing power may be secreted under the necessary stimulus. In the carious condition, however, there is no reserve power or, at least, where the factor is between 84 and 100, it is too small to be of much effect; in fact in those cases where the factor is above 100 per cent the neutralizing power of the activated saliva is less than that of the normal resting saliva. If it can be determined what physiological process is concerned in the production of an activated saliva, containing, constantly, a greater neutralizing power than the normal resting saliva, the writer ventures to suggest that a definite step will be made in the elucidation of the etiology of dental caries.

Henderson (19), in considering the equilibrium between acids and bases in the animal organism says that "This acid-base equilibrium may stand in direct relation to bone formation, rickets and osteomalacia." What the relationship may be between this equilibrium in the saliva and dental caries is indicated in the above tables.

SUMMARY

1. That the reaction of normal resting saliva is too variable to be a positive factor in indicating any of the following conditions:

(a) Absolute immunity either with or without care.

(b) Present immunity either with or without care.

(c) Caries either with or without care.

2. That a method analogous to that employed by Henderson (19) in measuring the power of the blood to maintain neutrality is applicable to the determination of the same power shown by the activated saliva.

3. By means of this method a "salivary factor" may be evaluated, namely, the ratio of the neutralizing powers of the "normal

resting" and "activated" salivas respectively, the magnitude of which appears to be indicative of immunity from caries or the reverse.

4. In persons who are either absolutely immune or for the present immune from caries the magnitude of this factor (expressed in percentage) varies between 43 and 80, while in persons whose teeth are carious this factor varies between 80 and 132.

The writer avails himself of this opportunity to express his appreciation and to acknowledge his indebtedness to those members of the faculty and heads of institutions who have shown such a whole hearted interest in the work presented and who have made it possible for him to carry out these experiments. Without their coöperation it would have been exceedingly difficult to obtain patients and to work out the several lines of thought suggested.

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THE INFLUENCE OF BLOOD TRANSFUSION ON THE HYPERGLYCEMIA AND GLYCOSURIA OF PANCREATIC DIABETES IN THE DOG

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In 1889 von Mering and Minkowski discovered that complete extirpation of the pancreas produces fatal diabetes.[1] The attempt of Pflüger to show that this diabetes following removal of the pancreas is due, not to the absence of the pancreas, but to injury to the duodenum and the nerves connecting the pancreas with the rest of the viscera, must be considered a failure.[2] The original conclusion of von Mering and Minkowski is definitely established: the loss of the pancreas results in fatal diabetes. But the subsequent endeavor to determine how absence of the pancreas causes diabetes is practically a record of repeated failures. The leading idea in all this work has been the internal secretion theory, or that the pancreas yields some substance to the blood in some way necessary for the oxidation of the sugar by the tissue cells. But in the absence of conclusive demonstration of the internal secretion the possibility that the work of the pancreas in maintaining normal sugar metabolism consists of detoxication processes must always be kept in view. The fact that even temporary glycosuria is not induced in normal animals by diabetic blood does not render the detoxication hypothesis untenable.

Some light has been thrown on the nature of pancreatic diabetes by results and experimental methods that may be inter-

[1] V. Mering and Minkowski: Arch. f. exp. Path. u. Pharm., 1889, xxvi, p. 371.

[2] Pflüger: Arch. f. d. ges. Physiologie, 1905, c. vi, p. 181; Minkoswki: Arch. f. exp. Path. u. Pharm., 1908, lix, p. 895.

preted either on the detoxication or the internal secretion theories. We refer to the methods of parabioses (Forschbach),[3] cross-circulation and transplantation (Hedon),[4] and pancreatectomy in late pregnancy (Carlson).[5] It is obvious that conditions which establish direct or indirect relation of the blood of a diabetic animal to living pancreas tissue may cause a change in the course of the disease either by detoxication or by internal secretion. On the other hand, if it can be shown that the oxidation of sugar by the tissues is augmented by some substance from the pancreas, or that pancreatic diabetes is temporarily ameliorated by transfusion of normal blood, the internal secretion theory is established.

The new method of attack introduced by Cohnheim[6] has not yielded consistent results, and in the light of the findings of Levene and Meyer the method itself is called in question, as it appears that in mixtures of sugar plus pancreas extract plus muscle extract, the sugar is polymerized, not oxidized.[7] The method of studying the action of pancreas extracts on the sugar consumption of the entire diabetic animal or on isolated but living organs of the diabetic animal is more physiological than that of Cohnheim, but the results to date are practically negative.[8] This may be due to failure to obtain the specific secretion in the pancreas extract, as well as to the difficulty of finding a reliable index of sugar oxidation in an isolated organ. In respect to this latter point it is obvious that our methods so far have been too crude or violent. The diabetic animal does not burn sugar, to any appreciable extent, which means that the individual organs of the diabetic animal do not burn sugar; yet when we compare the sugar consumption of the isolated organ of the

[3] Forschbach: Arch. f. exp. Path. u. Pharm., 1908, lx, p. 131.

[4] Hedon: Arch. intern. de. Phys., 1913, xiii, p. 4; p. 255.

[5] Carlson: Am. Jour. of Physiol., 1911, xxviii, p. 391; Jour. Biol. Ch., 1914, xvii, p. 19.

[6] Cohnheim: Zeitschr. f. physiol. Ch., 1903, xxxix, p. 336.

[7] Levene and Meyer: Jour. of Biol. Ch., 1911, ix, p. 389.

[8] Knowlton and Starling: Jour. of Physiol., 1912, xlv, p. 146; Maclean and Smedley: ibid., p. 420; Macleod and Pearce: Am. Jour. of Physiol., 1913, xxxii, p. 184; Patterson and Starling: Jour. of Physiol., 1913, xlvii, p. 137; Macleod and Pearce: Am. Jour. of Physiol., 1914, xxxiii, p. 378.

normal and the diabetic animal, we have so far failed to find any difference!

To our way of thinking the method least artificial and violent as regards the hypothetical pancreas secretion and the least injurious to the test object, that is, the diabetic animal, is the transfusion of normal blood. Yet, note the contradictory and ambiguous results from this mode of attack! Lepine reports a temporary diminution in the output of sugar in the urine, but no diminution in the blood sugar.[9] This would seem to point to some injurious action of the foreign blood on the kidneys. Hess injected intravenously 50-150 of blood from diabetic dogs into normal dogs (on the theory that diabetic blood might stimulate the pancreas to a greater output of the internal secretion) and 9–14 hours later he injected the serum from this animal into diabetic dogs. The influence on the glycosuria of the diabetic animal was slight or inconstant.[10] In view of the results reported from this laboratory a few years ago by Drennan,[11] it seems likely that in the experiments of Hess the pancreas secretion in the blood was destroyed by the delay in centrifuging the blood. Alexander and Ehrmann[12] injected blood from the pancreatic duodenal vein of normal dogs into diabetic dogs, but obtained no definite or constant decrease of the glycosuria. In some of the experiments secretin was added to the blood to be injected. The reason for this is not apparent. These investigators state that dogs with the pancreas completely extirpated but otherwise healthy and in good spirits sometimes void urine free from sugar, in the absence of all experimental interference. This has never been noted in this laboratory in the cases of more than 100 diabetic dogs, except in cases of incomplete pancreas extirpation.

Drennan, working in this laboratory, injected 50–150 cc. of fresh defibrinated dogs' blood into the veins of diabetic dogs and invariably obtained a temporary lowering of the urine sugar and

[9] Lepine: Le diobète sucré., 1909, p. 363.
[10] Hess: Münch. Med. Woch., 1902, p. 1449.
[11] Drennan: Am. Jour. of Physiol., 1911, xxviii, p. 396.
[12] Alexander and Ehrmann: Zeitschr. f. exp. Path., 1908, p. 367.

the D:N ratio. Defibrinated sterile blood loses this action on standing for a few hours. The course of the blood sugar in the injected animals was not studied. Hedon has reported a very extensive series of blood transfusion in diabetic dogs. Direct transfusion of normal blood into a diabetic dog previously bled dry causes a temporary lowering of the blood sugar and decrease or complete suppression of the glycosuria, but since the same results were produced when blood from a diabetic dog was transfused into another diabetic dog Hedon concludes that the temporary diminution of the hyperglycemia and glycosuria following the transfusion were not due to any specific pancreas secretion in the blood but to a lowering of the blood sugar by dilution and to a toxic action of the foreign blood on the kidneys. The results of the cross transfusion experiments reported by Hedon do not concern us here, since these may be interpreted in various ways (detoxication by the pancreas, storage of glycogen in the normal animal, dilution of the diabetic blood, etc.). Hedon also transfused (cross transfusion as well as serum injections) blood from the pancreatic vein of normal into diabetic dogs. A slight temporary lowering of the hyperglycemia with a greater reduction of the urine sugar was noted, but the latter is interpreted as due an injurious action of the foreign blood on the kidneys. Hedon concludes that the internal secretion of the pancreas acts on and is absorbed by the liver, and is therefore not present in the blood of the systemic circulation. Hedon attempted to obtain evidence in support of this view by introducing a living pancreas in the systemic and in the portal circulation of diabetic dogs. With the living pancreas interposed in the portal circulation the hyperglycemia and glycosuria were diminished, but interposed in the general circulation the pancreas had no effect. We do not think that these latter results of Hedon can be accepted, in view of what is known concerning the carbohydrate metabolism in dogs with Eck fistula. In the animal with the Eck fistula the internal secretion of the pancreas, if there is one, must pass into the general circulation, and only a small part of it can reach the liver by way of the hepatic artery, just as in Hedon's diabetic dogs with the living pancreas from

another dog interposed in the general circulation; yet the Eck fistula dog does not develop diabetes.

Murlin and Cramer[13] have recently reported one experiment with transfusion of normal blood into a diabetic dog, using the respiratory quotient as a measure of sugar oxidation. The average R.Q. for two one-hour periods before the transfusion was 0.678; for four one-hour periods after the transfusion 0.700. No conclusion can be based on the results of a single experiment, but so far as they go in this case, the transfusion raised the R.Q. Murlin and Cramer appear to have had in mind transfusion as "a measure of practical importance" in clinical diabetes, and are therefore disappointed with the results. To our way of thinking this one experiment was sufficiently encouraging to warrant ten or twenty additional ones, in the hope of settling the wexed question of internal pancreatic secretion, whether or not blood transfusion will be a measure of practical importance in clinical diabetes.

Raulston and Woodyatt appear to be the first to make use of blood transfusion as a practical therapeutic measure in diabetes melitus in man.[14] The patient was a man in the thirties, but the diabetes was of several years' standing, with severe acidosis, and periods of threatening coma. The blood (500 cc.) was yielded by a two year older brother of the patient. The sera of the two persons showed no lytic action on the respective corpuscles. *The blood transfusion augmented all the diabetic symptoms for several days.* In that respect this single transfusion test in clinical diabetes differs from all transfusion experiments in experimental diabetes so far in the literature. No investigator reports a single instance of blood transfusion aggrevating the pancreatic diabetes in dogs. In judging the importance of the negative results of this single test on man it must be taken into consideration that the patient was in the last stage of diabetes, and died in coma not long after the test. Furthermore, there is no evidence that this patient was primarily a case of pancreatic diabetes.

The above résumé goes to show that blood transfusion in

[13] Murlin and Cramer: Jour. of Biol. Ch., 1913, xv, p. 380.

[14] Raulston and Woodyatt: Jour. Am. Med. Ass., 1914, lxii, p. 996.

pancreatic diabetes has to date yielded positive (even if transitory), ambiguous or contradictory, and apparently negative results. This being the case, is there any justification for further attempts at analysis of the diabetes problem by the method of blood transfusion? We think there is and for the following reasons:

1. The results of transfusion experiments where the diabetic animal was bled dry or nearly dry before being transfused with normal blood are of doubtful value, because of the temporary asphyxia of the kidneys and the tissues in general by the excessive bleeding.

2. The results of prolonged cross transfusion or direct transfusion by means of direct connection of the blood vessels of donor and recipient are rendered practically valueless by use of anesthetics, or when anesthetics are not used, by the traumatism and struggling of donor and recipient.

3. Hedon's contention that the pancreatic internal secretion is found only in the portal circulation and that blood from the pancreas has no antidiabetic action unless introduced directly into the portal circulation is rendered untenable by the well known facts in the case of Eck fistula animals. It is probable that in Hedon's experiments the pancreas was too greatly traumatized or asphyxiated to function.

4. Negative results from injection of serum of normal animals into diabetic animals are of little or no significance, because the delay and manipulation of the blood to obtain the serum probably permits destruction by oxidation of most of the pancreatic secretion.

5. Extracts of the pancreas apparently injures the kidneys,[15] but Hedon's contention that transfusion of blood from one dog into another invariably injures the kidneys of the recipient in such a way that they become less permeable to sugar must be more firmly established, if it is to overthrow the positive results of transfusion in the way of decreased hyperglycemia and glycosuria.

[15] De Meyer: Arch. intern. de Physiol., 1909, viii, p. 121; Vahlen: Zeitsch. f. physiol. Ch., 1909, lix, p. 194.

6. Negative results of transfusion in advanced stages of absolute diabetes are probably of no significance as regards the question of an internal secretion of the pancreas, as in late diabetes secondary tissue changes are present, which the pancreas secretion is probably unable to modify directly.

EXPERIMENTAL PROCEDURE

The transfusion. In all of our experiments we aimed at transfusing a quantity of blood equal to about one-tenth the volume of the blood of the recipient. This was done without previous bleeding of the recipient. The blood was drawn from the carotid of the donor into a sterile syringe carefully oiled and injected into the recipient through a large hypodermic needle inserted into the saphenous vein. By working moderately fast there is no danger from coagulation. By this method the recipient is not subjected to anesthetics, traumatism, pain, or struggling. By transfusing only about one-tenth of the recipient's own blood volume there is no disturbance of the circulation from an excess of blood, and previous bleeding is therefore not necessary. It seems reasonable to suppose that the less disturbances the recipient is subjected to the more certain one can be in ascribing changes in the diabetes to some specific action of the transfused blood itself.

The determination of the blood sugar. The samples of blood for sugar determination were drawn from the saphenous vein by means of a hypodermic needle, or from the end of the tail. The quantity of blood drawn was determined by weighing. All the samples were approximately 5 cc. All our sugar determinations were made by the Rona-Michaelis' method, or a combination of this with the method of Bertrand. The tests were run in duplicates so far as this was possible.

The urine sugar was determined by the method of Benedict. The dogs were not catheterized as this cannot be done repeatedly without producing cystitis. Our 24-hour samples of urine are therefore subject to the time variations in voluntary micturition.

The care and feeding of the diabetic dogs. The pancreatectomized animals were fed on lean meat and water.

RESULTS

The influence of transfusion of normal blood on the hyperglycemia

The results of our 20 experiments on 12 diabetic dogs are given in Table I. In no case did the transfusion increase the diabetic hyperglycemia. In three experiments (Dog I, expt. 3, and Dog II, expt. 1; Dog V, expt. 1). There appéars to be no change in the blood sugar of the recipient. In all the remaining experiments there is a lowering of the hyperglycemia, but in two cases (Dog II, expts. 2 and 3) is too small to have any significance. The decrease in the hyperglycemia is very temporary, probably not exceeding 6–10 hours.

The experiments on Dogs VII–X demonstrate that this lowering of the hyperglycemia is not a matter of dilution of the diabetic with the normal blood, as the dilution factor must be at its maximum within a few minutes after completion of the transfusion. That dilution is not an important factor can be shown in other ways. For example, in a diabetic dog having 750 cc. of blood with a sugar content of 0.25 per cent transfusing 75 cc. of blood from a normal dog with the blood sugar 0.10 per cent can by dilution alone reduce the hyperglycemia from 0.25 per cent to 0.238 per cent. In this connection we may also note the results of three experiments in which we made intravenous injections of Ringer's solution in amounts equal to one-tenth of the total quantity of blood in the diabetic dog:

Experiment 1. Blood sugar before transfusion 0.34; blood sugar 3½ hrs; after transfusion 0.30.

Experiment 2. Blood sugar before transfusion 0.25; blood sugar 2½ hrs; after transfusion 0.25.

Experiment 3. Blood sugar before transfusion 0.25; blood sugar 2¼ hrs; after transfusion 0.26.

Since the output of sugar in the urine in diabetes is proportional to the hyperglycemia, it is evident that the temporary reduction of the hyperglycemia of pancreatic diabetes by transfusion of normal blood is a factor in the parallel reduction of the glycosuria.

TABLE I.

The influence on the hyperglycemia of pancreatic diabetes in dogs of transfusion of blood from normal dogs. The blood sugar of the donors varied from 0.07 per cent to 0.10 per cent. The last column gives the time after the transfusion when the samples of blood were drawn.

DOG	EXPERIMENT	BLOOD SUGAR		TIME AFTER TRANSFUSION
		Before trans.	After trans.	
I	1	0.23	0.15	2½ hrs.
	2	0.23	0.14	1¾ hrs.
	3	0.17	0.17	2½ hrs.
II	1*	0.12	0.12	2½ hrs.
	2	0.21	0.19	5 hrs.
	3	0.33	0.30	5 min.
			0.32	2 hrs.
III	1	0.32	0.15	3 hrs.
	2	0.30	0.26	4½ hrs.
IV	1	0.23	0.15	5½ hrs.
	2	0.20	0.17	1¾ hrs.
	3	0.19	0.16	4½ hrs.
V	1†	0.17	0.17	4¾ hrs.
	2	0.25	0.22	10 min.
			0.20	2½ hrs.
VI	1	0.26	0.16	10 min.
			0.19	1¾ hrs.
VII	1	0.25	0.20	5 min.
			0.12	1 hr.
			0.17	2½ hrs.
			0.28	24 hrs.
VIII	1	0.29	0.25	5 min.
			0.21	1⅛ hrs.
			0.15	3¾ hrs.
IX	1	0.29	0.25	5 min.
			0.21	1 hr.
			0.25	2½ hrs.
X	1	0.25	0.23	6 min.
			0.19	1¼ hrs.
			0.19	4 hrs.
			0.23	24 hrs.
XI	1	0.29	0.26	5 min.
			0.27	2¼ hrs.
XII	1	0.35	0.28	5 min.
			0.26	2½ hrs.

* Sandmeyer type of diabetes owing to trace of pancreas left on duodenum.

† In this experiment the donor was an old dog with adenomatous cancer of the thyroid.

The influence of the transfusion of normal blood on the glycosuria of pancreatic diabetes

The results of our 16 experiments on 9 dogs are summarized in Table II. The data in this table seem to warrant the following conclusions:

1. The glycosuria is in no case augmented by the transfusion.

TABLE II.

The influence of transfusion of normal blood on the glycosuria of pancreatic diabetes in dogs. The 24 hours volume of urine is only approximate, as the dogs were not catheterized.

DOG	EXPERIMENT	24 HOURS VOLUME OF URINE			24 HOURS OUTPUT OF URINE SUGAR		
		Day before transfusion	Day of transfusion	Day following transfusion	Day before transfusion	Day of transfusion	Day following transfusion
		cc.	*cc.*	*cc.*	*gr.*	*gr.*	*gr.*
	1	640	560	510	20.50	12.300	17.34
I	2	680	660	680	21.48	14.570	19.65
II		380	187	160	10.00	2.390	7.50
III	1	234	176	220	16.38	6.840	17.80
	2	173	107	250	14.60	3.780	16.85
IV	1	500	630	450	10.20	5.300	10.26
	2	450	195	500	12.40	2.140	10.10
V		185	160	170	3.60	1.150	3.95
VI	1	250	220	204	16.42	3.760	10.62
	2	160	130	170	8.96	0.557	3.74
VII	1	650	560	490	18.98	5.000	17.53
	2	485	450	400	15.37	3.200	10.00
VIII	1	625	980	860	22.80	6.300	28.90
	2	550	653	655	16.42	7.500	16.60
IX	1	540	377	376	13.00	5.440	6.68
	2	353	261	540	10.57	3.900	13.10

2. The transfusion diminishes the glycosuria, but in no case is the urine rendered sugar free.

3. In every case when frequent samples of urine were obtained after the transfusion the lowering of the urine sugar lasted only 4–10 hours after the transfusion, that is to say, the depression of the glycosuria runs parallel with the lowering of the hyperglycemia.

4. In most cases there is some depression of the polyuria parallel

with the decreased sugar output, but the effect of the transfusion appears most strikingly in the percentage of the urine sugar.

5. There is no evidence that the sugar retained by the dogs for the first 24-hour period following the transfusion is eliminated as excess sugar during the subsequent 24 hours. The reader may note that there is a slight indication of such a possibility in experiment 1, Dog VIII, but in some of the other experiments the relations are exactly reversed. These small fluctuations in either direction are therefore of no significance.

The influence of transfusion of diabetic blood on the hyperglycemia of pancreatic diabetes

The reader will recall that according to Hedon transfusion as such produces some temporary injury to the kidneys in consequence of which the output of sugar in the urine is temporarily diminished. Hedon reports the same results from transfusion of diabetic blood as from transfusion of normal blood. With our method of transfusion we have been unable to confirm Hedon on this point. Our ten experiments on six dogs are summarized in Table III. The transfusion (according to our method) of blood from a dog in complete pancreatic diabetes has no effect on the hyperglycemia of the latter. This fact indicates that the

TABLE III.

Transfusion of blood from dogs in pancreatic diabetes into dogs in pancreatic diabetes. The transfusion does not influence the hyperglycemia of the recipient.

DOG	BEFORE TRANSFUSION	AFTER TRANSFUSION	TIME
			hours
I*	0.120	0.120	3
II	0.210	0.210	2
	0.260	0.270	2
	0.280	0.280	2
III	0.240	0.235	2
	0.237	0.267	2
IV	0.310	0.308	2
	0.270	0.255	2½
V	0.210	0.220	1
VI	0.300	0.290	3

* Dog with incomplete diabetes (Sandmeyer type).

lowering of the hyperglycemia of pancreatic diabetes by the transfusion of normal blood is not due to depression of the tissues in general and hence lowering of the sugar production.

The influence of transfusion of normal blood into normal dogs on the sugar tolerance of the recipient

The suggestion of Hedon that blood transfusion as such injures the kidneys in such a way as to temporarily diminish sugar excretion is so important for the interpretation of all results obtained by transfusion methods that it should be tested in all possible ways before it is finally rejected, or accepted. The following tests on a series of normal animals were accordingly carried out. Seventeen dogs were injected intravenously with 1.5 gram dextrose (20 per cent solution) per kilo body weight and the quantity of sugar excreted during the following 24-hour period determined. Another series of nineteen dogs were transfused with normal blood by the same method employed in our diabetic dogs before receiving corresponding quantities of dextrose intravenously. If this blood transfusion injures the kidneys in such a way as to temporarily decrease their capacity to excrete sugar we would expect less sugar in the urine of these dogs, as a normal dog is capable of storing and burning large quantities of sugar. Our results did not come out in that way. *The average total output of sugar in the urine was 0.96 gram for the control group and 1.33 gram for the transfused group.* It is well known that there is considerable individual variations in the sugar tolerance in dogs, whether tested by the hypodermic, the intramuscular, or the intravenous methods, and both of our series showed these individual fluctuations. We therefore deemed it necessary to use the above great number of dogs in order to neutralize this source of error as far as possible.

In normal dogs and with normal blood, our method of transfusion does not impair the power of the kidneys to excrete sugar during hyperglycemia. So far as the transfusion has any effect at all it appears to increase the sugar excretion. But the final establishment and explanation of this latter point requires further investigation.

The influence of blood transfusion on normal and diabetic dogs on the general secretory activity of the kidneys

It seemed to us, that if blood transfusion as such injures the kidneys sufficiently to depress their power to excrete sugar, such injury ought to appear in the capacity of the kidneys to excrete some or all of the normal constituents of the urine. The work on this phase of the general question was done in our laboratory by Mr. I. Rabens, and the detailed results will be reported later. In normal and in pancreatectomized dogs on standard rations of lean meat and fixed quantities of water, the total nitrogen, urea, ammonia, amino acids, phosphorus, chlorides and (in the diabetic dogs) sugar were determined for 24-hour periods before and after transfusion with normal blood. In the normal dogs the transfusion had no effect on the kidney activity; in the diabetic dogs the transfusion diminished only one of the above constituents, namely the sugar.

It would therefore seem that our method of blood transfusion has no demonstrable injurious action on the kidneys either in normal or in diabetic dogs. Hedon's explanation of the effect of blood transfusion on the glycosuria and hyperglycemia of pancreatic diabetes is therefore untenable, at least as regards our results.

DISCUSSION OF THE RESULTS

The transfusion of normal blood into dogs in pancreatic diabetes causes a temporary lowering of the hyperglycemia. But before this can be taken as an evidence of the presence in normal blood of an internal secretion from the pancreas, it must be shown that this temporary lowering of the hyperglycemia is due to storage and oxidation of sugar in the tissues. Our work indicates that sugar retained as a result of the blood transfusion is not subsequently excreted. If this is the case what can happen to this sugar except oxidation in the tissues? In the first place, a temporary lowering of the rate of tissue metabolism might account for the temporary fall in blood sugar. Or, the metabolism rate being the same, some of the sugar may disappear by

way of the digestive secretions[16] and bacterial oxidation in the digestive tract. However improbable these possibilities may seem, they must be reckoned with in the absence of definite proof that this sugar is oxidized by the diabetic tissues.

SUMMARY

The transfusion of normal blood into dogs in complete pancreatic diabetes, by the method outlined above, causes a temporary (4–8 hours) lowering of the hyperglycemia and the glycosuria.

Similar transfusions of diabetic blood into diabetic dogs have no effect on the hyperglycemia.

There is no indication in our results that the sugar retained by the animal in consequence of this temporary lowering of the sugar excretion by the kidneys is subsequently eliminated by the kidneys as excess sugar.

The blood transfusion as such does not impair the kidneys' activity in any demonstrable way, either in diabetic or in normal dogs. The temporary lowering of the glycosuria of pancreatic diabetes by transfusion of normal blood is due to the diminished hyperglycemia, not to kidney injury.

[16] Carlson and Ryan: Am. Jour. of Physiol., 1908, xxi, p. 301.

THE INFLUENCE OF BLOOD TRANSFUSION ON THE KIDNEYS

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Hedon[1] reports that transfusion of normal as well as diabetic blood into diabetic dogs leads to a temporary decrease in the glycosuria. According to Hedon the foreign blood received by the diabetic animal is in some way injurious to the kidney, and the lowered glycosuria following a transfusion is not due to introducing blood containing the internal secretion which would temporarily help the tissues to utilize the sugar in the blood, but to a decreased permeability of the kidney to sugars through the injurious action of the foreign blood, normal or diabetic.

Carlson and Ginsburg[2] found a lowered glycemia accompanying the decreased glycosuria after transfusion of normal blood into diabetic dogs. The reduced sugar content of the urine could thus be accounted for by the reduced sugar content of the blood without any injurious action on the kidneys as claimed by Hedon. Moreover, they found no change in the glycemia or glycosuria following transfusions with diabetic blood. The fact that Hedon obtained a reduction in the glycosuria after transfusion with diabetic blood they believed to be due to his severe method of transfusion. They also tested the sugar tolerance of normal dogs before and after transfusion with normal blood, and found no increased tolerance after transfusion as would be expected if the foreign blood produced a decreased permeability of the kidneys to sugar.

At the suggestion of Dr. Carlson the following work was undertaken as an additional test of the possible injurious action of

[1] Hedon, Arch. internat. de physiol., 1913, xiii, p. 4, p. 255.

[2] Carlson and Ginsburg, This Journal, 1915, xxxvi, p. 280.

transfusion on the kidneys in normal and in diabetic dogs. It would seem that if blood transfusion had any direct effect on the kidneys this should be disclosed by a quantitative determination of the principal urinary constituents eliminated for 24-hour periods by both the normal and diabetic animal before and after blood transfusion.

This was the primary object of the work. Indirectly the results of these tests may also throw light on the effects on the kidneys of blood transfusion in man. Transfusion is coming into greater vogue clinically, especially in the spontaneous infantile hemorrhages, in anemias, in shock, where at least a temporary rise of blood pressure is looked for, and in septicemias, where an increased supply of antibodies is desired.

EXPERIMENTAL PROCEDURE

The dogs were kept in ordinary metabolism cages, and fed daily on a definite quantity of boiled fresh beef-heart freed from fat as completely as possible. They were also given a definite quantity of water, once daily, and generally by a stomach tube.

The transfusions were made for me by Dr. Carlson and Mr. Ginsburg according to the method employed by them in the previous work. A fairly large young dog was selected to serve as donor. This was done to provide against the possibility of a spontaneous nephritis often observed in old dogs in this region.[3] The amount of blood injected was calculated to approximate roughly one-seventh to one-tenth of the entire blood content of the recipient.

The quantitative determinations were made of the following urinary constituents. The determinations were made in duplicate to guard against any possible error in technique.

1. *Total nitrogen* by the Kjeldahl method as modified by Gunning and Arnold.

2. *Total urea* by the Marshal urease method (modified by F. C. Koch).

3. NH_3 by the Folin aeration method (modified by F. C. Koch).

[3] Personal communication from Dr. C. E. King.

4. *Amino acids* estimation by the Formol Titration.
5. *Total phosphorus* by the Neumann-Pemberton Method.
6. *Total chlorides* by the modified Volhard Method.
7. *Reducing sugars* by the Munson-Walker and Bertrand method.
8. *Total acidity and specific gravity.*

In the diabetic dogs the urine sugar was determined on every sample of urine voided the first 24 hours following the transfusion. The estimation of the other constituents were made on the 24-hour sample. In the calculations both the total and percentage of each constituent were figured out, for the volume of urine varied somewhat from day to day. Eight dogs were used in this work, 10 transfusions being made on normal, and 5 on diabetic animals.

RESULTS

Our results fail to bring out any evidence of injury or depression of the kidneys by Carlson's method of transfusion, as will be seen by examination of the three typical sets of experiments summarized in Tables I to III. There are some variations in all the urine constituents from day to day, but with the exception

TABLE I

Normal dog No. II. Weight, 10 kgm.; receiving 300 grams boiled beef heart daily. Analysis of the urine for three days before (control) and three days following transfusion of 75 cc. of blood from another dog

	3 DAYS CONTROL PERIOD			3 DAYS TRANSFUSION PERIOD		
	1	2	3	1	2	3
Volume of urine, cc....	325.	300.	325.	350.	400.	290.
Sh. sp. gr.....	1034.	1026.	1025.	1026.	1021.	1030.
Acidity (in cc. of $\frac{N}{10}$ NaOH per 100 cc.)	97.5	68.0	61.2	81.0	54.50	74.8
Total nitrogen, grams..	10.000	7.560	7.190	8.800	8.230	9.110
Amino acid nitrogen, grams.....	0.406	0.353	0.375	0.376	0.336	0.461
Urea, grams.....	10.450	14.040	10.350	11.390	11.060	7.680
Ammonia, grams.....	0.371	0.365	0.456	0.497	0.443	0.507
Chlorides, grams.....	0.247	0.213	0.345	0.423	0.487	0.494
Phosphorus, grams....	0.800	0.610	0.610	0.720	0.680	0.740

TABLE II

Dog VI. Weight, 8 kgm. Dog in complete pancreatic diabetes. Analysis of urine for 24 hours before and for two days following transfusion of 100 cc. blood from a normal dog. Dog fed 200 grams boiled beef heart daily

	BEFORE TRANSFUSION 24 HOURS	AFTER TRANSFUSION	
		1st day	2d day
Volume of urine	350.	400.	300.
Specific gravity	1044.	1024.	1043.
Acidity (in cc. of 0.1 NaOH per 100 cc.)	80.	46.	68.
Total nitrogen, grams	8.040	7.840	6.000
Amino acid nitrogen, grams	0.580	0.480	0.510
Urea, grams	7.240	5.820	5.220
Ammonia, grams	0.530	0.560	0.510
Chlorides, grams	0.015	0.025	0.011
Phosphorus, grams	0.430	0.500	0.480
Sugar, grams	19.180	10.520	17.480

TABLE III

Dog V. Weight, 9.7 kgm. Dog in complete pancreatic diabetes. Analysis of the urine for 24 hours before and for two days following transfusion of 100 cc. of blood from a normal dog. Dog given 200 grams boiled beef heart daily

	BEFORE TRANSFUSION 24 HOURS	AFTER TRANSFUSION	
		1st day	2d day
Volume of urine	350.	320.	350.
Specific gravity	1043.	1033.	1046.
Acidity	57.	41.	60.
Total nitrogen, grams	6.930	5.220	7.060
Amino acid nitrogen, grams	0.562	0.466	0.592
Urea, grams	8.210	4.250	6.360
Ammonia, grams	0.600	0.550	0.710
Chlorides, grams	0.017	0.091	0.025
Phosphorus, grams	0.520	0.470	0.650
Sugar, grams	17.500	14.000	16.500

of the urine sugar in the case of the diabetic dogs, these daily variations are not definitely related to the transfusion. There are also fluctuations in the percentage of the urine sugar in the diabetic dogs, but these do not account for the characteristic lowering of the glycosuria by the transfusion (Table IV). In 1 out of the 5 transfusion experiments in the diabetic dogs, there is a slight diminution in practically all of the urine constituents

for the first 24 hours following the transfusion (see Table III). The other four groups of the diabetic dogs are practically identical with the one given in Table II. It is therefore clear that the temporary decrease in the glycosuria following these transfusions cannot be accounted for by kidney injury. So far as can be determined by examination of the urine this transfusion has no effect on the kidneys or on the general level of the body metabolism.

TABLE IV

Diabetic dog V. Sugar determinations of every sample of urine voided for 24 hours before and for 24 hours after transfusion of dog VI (see Table II)

BEFORE TRANSFUSION			AFTER TRANSFUSION		
Time	Sugar	Sugar	Time	Sugar	Sugar
hours	*grams*	*per cent*	*hours*	*grams*	*per cent*
23	2.72	5.33	2	2.20	2.50
20	2.42	5.50	3½	1.48	2.64
17	2.59	6.47	5¼	1.40	1.91
12½	1.75	5.73	9½	1.15	1.79
2	7.85	5.49	12	2.04	4.16
			21	2.64	3.38
Total.....	19.18	5.48	Total.....	10.52	2.65

The reader will note the very low output of total chlorides in diabetic dogs. This was a uniform result. On the same diet the diabetic dogs eliminated in the urine only about one-seventh to about one-tenth of the chlorides found in the urine before pancreatectomy. Are these chlorides eliminated in the feces? Or, are they retained in the tissues? These dogs showed no evidence of edema, and Dr. Carlson informs me that he has never observed any tendency to edema in pancreatic diabetes in dogs. The cause of this lowered output of urine chlorides in pancreatic diabetes is being further investigated in this laboratory.

We desire to express our hearty gratitude to Drs. A. J. Carlson and F. C. Koch for their supervision and advice while this work was in process.

THE THRESHOLD STIMULUS OF THE CHORDA TYMPANI NERVE IN RELATION TO SALIVARY SECRETION AND VASODILATION

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A vast amount of work has been done on the chorda tympani nerve in regard to secretion and vasodilation, but no accurate determination of the threshold stimulus of this nerve as far as I am aware has yet been made. In some cases the investigators attempted to obtain some idea of how strong a current is necessary to produce a secretion of saliva or to bring about dilation of the vessels in the submaxillary gland supplied by this nerve. They employed the method of moving the secondary coil a certain distance from the primary coil without taking into consideration tissue resistance. Since it is only recently that a reliable method for determining the strength of faradic stimuli has been worked out, it is not surprising to find very little literature directly pertaining to the subject here involved.

The present investigation was undertaken (1) to determine what is the weakest stimulus required by the chorda tympani (a) to produce a secretion of saliva, (b) to produce dilation of the vessels supplied by this nerve and (2) to determine whether or not a difference exists in the threshold stimuli necessary to produce these phenomena.

THE METHOD

Dogs, anaesthetized with ether and quickly decerebrated, were employed in these experiments.[1] The skin was cut on the median line of the neck and the mylo-hyoid muscle cut and laid

[1] Gruber: This Journal, 1913, xxxii, p. 438.

back exposing the chorda tympani nerve and the submaxillary duct. The nerve was isolated and freed from as much fascia as possible. A glass electrode[2] was placed on the nerve so that the cathode was proximal to the gland. Stretching and unnecessary handling of the nerve were avoided.

The method used to indicate the presence of salivary secretion was very simple. A cannula was inserted in Wharton's duct. As the saliva was secreted it fell in drops upon a lever attached to a receiving tambour, which, in turn, was connected to a recording tambour. When very weak currents—those nearing the threshold—were used direct observation of the saliva accumulating at the end of the cannula was necessary.

In much the same manner vasodilation was recorded. A cannula was placed in the external jugular vein after all the veins contributing to it, save the one supplying the submaxillary gland, had been tied off. The rate of blood flow was recorded in drops. Each drop, falling on a lever attached to an electro-magnetic signal, broke the circuit, thus causing a mark to be made on the surface of the drum by the signal. Later tambours were used instead—a receiving and a recording tambour—like those used in recording salivary secretion.

The threshold stimulus was measured by means of the Martin method in which the strength of stimulus is calculated in β units.[3] The position of the secondary coil, in every case, was read by moving it away from the primary coil until the smallest amount of vasodilation or of secretion of saliva occurred. Four of these readings were made, one with tissue resistance and the others with 10,000, 20,000 and 30,000 ohms resistance in the secondary circuit. Immediately after the determination of the position of the secondary coil, before the electrodes were disturbed in any way, three readings of tissue resistance were made.[4]

The strength of the primary current for determining the threshold was either 0.1 or 0.2 amperes. The rate of stimulation was usually five per second. This rate was chosen because it was

[2] Sherrington: Journal of Physiology, 1909, xxxviii, p. 382.
[3] Martin: Measurement of Induction Shocks, New York, 1912, pp. 60–69.
[4] Martin: *Loc. cit.*, pp. 71–93.

found favorable to produce dilation of the vessels supplying the extremities,[5] it seemed therefore probable that it might prove favorable in the production of dilation of the vessels supplied by the chorda tympani. Slower and more rapid rates were also employed. The inductorium which was used throughout had a secondary resistance of 2400 ohms. This was added to the average tissue resistance in making corrections. Corrections were also made for core magnetization. In this coil the value of K was 0.22.[6]

Carlson found it possible upon stimulating the cervical sympathetic to obtain, in some cats, marked dilation of the vessels without secretion of saliva.[7] Such was not the case, I found, upon stimulation of the chorda tympani. In the experiments here performed the strength of stimulus necessary to produce secretion also produce vasodilation in every case and vice versa. Table 1 shows the thresholds thus obtained. The Z units varied from 2.2 to 12.80 or an average for the sixteen experiments of 5.4 Z units. β units were calculated each time and these varied from 1.6 to 7.58 or an average for the sixteen experiments of 3.14 β units (see table 1).

The results here obtained are interesting in that they agree closely with those obtained upon stimulation of the cervical sympathetic in cats. Mendenhall found the threshold of this nerve for constriction of the vessels of the nasal membrane to be 7.89 Z units and 4.58 β units.[8] Still more striking is the similarity between the threshold of the chorda tympani and the threshold of the cervical sympathetic when the average of the thresholds (pupil, nictating membrane, and nasal vasoconstrictors) of the latter nerve is taken—6.65 Z units and 3.86 β units. This similarity can easily be understood if we consider the fact that a ganglion is traversed in each case. Langley has shown the presence of nerve cells within the hilus of the submaxillary gland

[5] Bowditch and Warren: Journal of Physiology, 1886, vii, p. 416; Bradford: *ibid.*, 1889, x, p. 390.

[6] Martin: *Loc. cit.*, p. 46.

[7] Carlson: This Journal, 1907, xix, p. 409.

[8] Mendenhall: This Journal, 1914, xxxvi, p. 60.

itself,[9] which cells bear the same relation to the chorda tympani as does the superior cervical sympathetic ganglion to the cervical sympathetic nerve.

It can be seen, upon comparison, that the thresholds of the chorda tympani and the flexion reflex of the hind leg of a cat, are markedly similar. Porter found 5.2 Z units and 2.7 β units necessary to arouse this reflex.[10] In my experiments on the

TABLE I

The threshold stimulus of the chorda tympani nerve as shown by the dilation of the vessels in the submaxillary gland and by the secretion of saliva

Z UNITS	β UNITS	RATIO OF β TO Z
2.20	1.60	0.73
3.65	1.83	0.50
3.65	1.60	0.44
3.65	3.20	0.90
4.17	2.06	0.50
4.17	2.40	0.58
4.20	2.40	0.57
4.38	1.74	0.39
4.59	2.03	0.43
4.59	2.90	0.63
4.59	2.86	0.62
4.59	3.26	0.71
5.22	2.20	0.42
6.37	4.44	0.70
7.94	2.96	0.37
11.90	8.80	0.74
12.80	7.58	0.59
Average.... 5.45	3.14	0.58

peroneus communis nerve and the tibialis anticus muscle (nerve muscle preparation) I found that the threshold stimulus was 1.2 β units.[11] Porter found the threshold for a simple nerve muscle to be 1.4 β units.

For thirty-one experiments Porter obtained a ratio of β to Z of 0.57.[12] Mendenhall found the ratio for his twelve experi-

[9] Langley: Journal of Physiology, 1890, xi, p. 125.
[10] Porter: This Journal, 1912, xxxi, p. 148.
[11] Gruber: *Loc. cit.*, p. 477.
[12] Porter: *Loc. cit.*, p. 148.

ments to be 0.58.[13] In these sixteen experiments the ratio for the average β to Z is 0.58. This, however, is merely a coincidence because if any experiment is omitted from the series, the ratio is changed. For example if the last is omitted then the ratio is 0.52 instead of 0.58. It has been suggested recently that this ratio may be taken as a quick means of calculating β units for a whole series of experiments. Upon this suggestion I reviewed my experiments upon the thresholds of the peroneus communis nerve and the tibialis anticus muscle. It may be well to give the

TABLE II

The average thresholds obtained upon the peroneus communis nerve and the tibialis anticus muscle

	AVERAGE Z	AVERAGE β	RATIO OF AVERAGE β TO Z	NUMBER OF EXPERIMENTS
Nerve muscle	1.78*	1.18	0.63	16
	3.90*	3.08	0.78	10
	1.33†	0.80	0.60	8
Muscle directly	25.82*	18.80	0.73	15
	40.26*	29.85	0.74	10
	23.50†	18.70	0.79	8
	28.40‡	25.00	0.74	8
Denervated muscle	150.50‡	61.40	0.40	6
Chorda tympani	5.45	3.14	0.58	17

* Gruber: This Journal, 1913, xxxii, p. 477; *ibid*, p. 448.
† *Ibid*, 1914, xxxiii, p. 338.
‡ *Ibid*, 1914, xxxiv, p. 92.

average Z units and the average β units of those experiments in this connection (see table 2).

From table 2 it can be seen that a wide variation exists in the ratios of β to Z in the several series of experiments performed. If however, we take the ratio of the average β to the average Z it will be 0.57. But if an average of the ratios is made it will be 0.66. Moreover, since the value of β depends upon the tissue resistance as well as upon the Z units it is quite important that the tissue resistance in all cases be the same if β is to be calculated

[13] Mendenhall: *Loc. cit.*, p. 60.

in this way. In table 1 we have three examples which show variations in β's calculated from like Z's with different tissue resistances, e.g., two Z values of 4.17 with resulting β units of 2.0 and 2.4, two Z values of 3.65 with β units of 3.20 and 1.6, and three Z units of 4.59 with β units of 2.03, 3.26 and 2.9. Porter shows a marked difference between ratios of single β's and their Z's. These range from 0.23 to 0.92 with an average ratio of 0.59 for the seventeen experiments on the flexion reflex and from 0.13 to 0.83 with an average ratio of 0.55 for the fourteen experiments on the nerve muscle.[14] From these results it is quite evident that the Martin formula or the formula established by Wilbur must be employed to calculate the β units accurately even for a long series of experiments.[15]

A few experiments were performed by employing different rates of stimulation. The threshold stimulus remained the same when the rate was from 3 to 15 per second. As the rate was decreased below 3 per second the threshold increased. It was possible to bring about a secretion by a single make or break shock but the strength of current necessary was greatly in excess of the threshold stimulus.

SUMMARY

1. The threshold stimulus for dilation of the vessels in the submaxillary gland and the secretion of saliva of this gland are the same.

2. The rate of stimulation within certain limits (3 to 15 per second) does not influence the thresholds of vasodilation and salivary secretion. For lower rates an increase in the threshold stimulus is necessary to bring about these changes.

3. The average threshold stimulus of the chorda tympani was found to be 5.4 Z units and 3.14 β units. This threshold is practically the same as that found by Mendenhall for the cervical sympathetic nerve.

4. A fixed ratio of β to Z cannot be used to determine accurately the β units of a series of experiments of any length.

[14] Porter: *Loc. cit.*, p. 148.

[15] Martin: *Loc. cit.*, p. 60; Martin Bigelow and Wilbur: This Journal, 1914, xxxiii, p. 416.

FACTORS AFFECTING THE COAGULATION TIME OF BLOOD

VI. The Effect of Rapid Progressive Hemorrhage upon the Factors of Coagulation

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It is an old observation that progressive bleeding decreases the coagulation time of the blood. Gray and Lunt[1] have summarized the literature on this subject. We will, however, review briefly the chief explanations which have been offered for this decrease in coagulation time.

Hewson[2] in 1780 attributed the increased coagulability to a probable change in "that state of the blood vessels on which the thinness and lessened tendency of the lymph to coagulate depends; which surely is a very curious circumstance." Nasse[3] in 1842 and Brücke[4] in 1857 confirmed the observation as to the increased coagulability but showed at the same time a decrease in the fibrin content of the blood. This fact led them to conclude that the fibrin content and the rate of coagulation of the blood do not necessarily run parallel. Milian[5] in 1901 making his observations on hemorrhage following capillary puncture, thought that the increased coagulability was due to a local accumulation of a coagulating substance, stored in the skin and

[1] Gray, H. and Lunt, L. K.: American Journal of Physiology, 1914, xxxiv, 332.

[2] Hewson, W.: Experimental Inquiries into the Properties of the Blood, 1780, Part I, Experiment XX, 55.

[3] Nasse, H.: Handwôrterbuch der Physiologie (Wagner), 1842, I, 75.

[4] Brücke, E.: Archiv für Pathologische Anatomie und Physiologie und für Klinische Medizin (Virchow), 1857, xii, 81, 172.

[5] Milian, G.: Mémoires de la Société de Biologie, 1901, liii, 556, 576.

in the tissues and released when they were wounded. Arloing[6] in the same year, experimenting with venous hemorrhage and finding the same shortening of coagulation time, opposed Milian's view of a local secretion of coagulating substance by the cells of the skin and tissues but suggested the possibility of an increase in fibrin ferment, resulting from an alteration in the blood when it came in contact with the lumina of the tubes which carried it from the veins to the receiving flasks. Arthus[7] in 1902 suggested that the acceleration of the coagulation time might be due, not to a quantitative increase in the amount of fibrin ferment in the blood after hemorrhage, but to an acceleration of its production. Hartman[8] in 1909 was unable to choose between diminished oxygen, augmented carbon dioxide, augmented fibrin ferment, and augmented flow of tissue thrombokinase. Von den Velden[9] in the same year explained the decreased coagulation time as the result of an augmented thrombokinase which reached the blood stream through an influx of tissue juice, and confirmed the observation of Nasse and Brücke that an immediate decrease in the fibrin content of the blood accompanies the decrease in coagulation time.

In order to throw further light on this interesting problem and to determine whether or not a quantitative alteration in the different factors of coagulation corresponding to a change in coagulation time might be observed, we undertook the series of experiments which are reported in this paper. In each experiment determinations of the coagulation times and analyses of the factors of coagulation were made before and after successive hemorrhages.

I. METHODS OF WORK

The technique of obtaining and examining the specimens of blood and of determining the coagulation times was as follows. The test animal was first anaesthetized with urethane (2 grams

[6] Arloing, F.: Mémoires de la Société de Biologie, 1901, liii, 675.

[7] Arthus, M.: Journal de Physiologie et de Pathologie Générale, 1902, iv, 273.

[8] Hartman, J.: Münchener Medizinische Wochenschrift, 1909, lvi, 796.

[9] Von den Velden, R.: Archiv für Experimentelle Pathologie und Pharmakologie, 1909, lxi, 37.

per kilo). The femoral or in some cases the carotid artery was next exposed, ligated distally and clamped proximally. A glass cannula washed with oxalate solution was then slipped into an oblique cut in the artery but not tied, the clamp was released, and the blood allowed to flow from the cannula. Blood obtained in this way supplied the specimens to be analyzed for the factors of coagulation and the specimens upon which coagulation time was determined. The specimens to be analyzed for the factors of coagulation were collected in glass graduates containing 1 per cent sodium oxalate in a 0.9 per cent sodium chloride solution in the proportion of 1 cc. of oxalate solution to 8 cc. of blood. A few drops of blood were first allowed to drop into a graduate containing the oxalate; the specimens upon which the coagulation time was determined were next collected in two test tubes (diameter 10 mm.), each graduated to hold 1 cc.; after which procedure the collection of the specimen to be analyzed was completed. The purpose of this order of work was to obtain for the determination of coagulation time a specimen of blood which had not stood in contact with the injured vessel wall and yet one which represented the condition of affairs in the body before the bulk of the hemorrhage occurred. The amount of the hemorrhage varied in different animals depending on their size. At the end of the bleeding the vessel was clamped proximally and the cannula removed.

As soon as the blood for determining coagulation time was collected, the tubes were placed in a water bath and kept at 37°–38°C. until they could be inverted without dislodging the clots. Observations were made by gently tilting the tubes at the end of five minutes and then at the end of every minute. Each coagulation time was taken with a stop-watch which was started as soon as the blood began flowing into the tube.

At an interval—usually of twenty minutes—the procedure described above was repeated, and a second determination of coagulation time (represented by the average of the two tubes) was made, and a corresponding oxalated specimen obtained to be analysed for the factors of coagulation. A fresh cannula or the old cannula freshly washed was used. This process of

bleeding at intervals of about twenty minutes was continued until the animal died. The later hemorrhages were of course smaller than the earlier ones, but varied in amount in different experiments.

After the death of the animal the specimens of oxalated blood were centrifugalized for 10 minutes at high-speed, and the plasma pipetted off. A portion of the plasma from each specimen was used to make *prothrombin* determinations; a portion was used to make *antithrombin* determinations; and a third portion (10 cc. if possible) was used to make *fibrinogen* determinations. In five experiments platelet counts[10] were made before each hemorrhage.

The methods of making prothrombin and antithrombin determinations were those described by Howell[11] and reviewed by Drinker and Hurwitz in an article not yet published. The fibrinogen determinations were made by the heat coagulation method.

II. EXPERIMENTAL DATA

Nine experiments were done on cats and eight on rabbits. In every experiment in which the coagulation time as a whole decreased there was a decrease in the amount of antithrombin. In all but two cases there was a steady fall in the fibrinogen content. The behavior of the prothrombin was irregular, on the whole tending first to increase slightly in amount and then to decrease. This irregularity in the prothrombin reaction depends to a certain extent upon the variations in antithrombin. With a fall in antithrombin one would expect a relative increase in prothrombin, though the absolute amount of the substance remained constant. In view of this fact the slight relative fall in prothrombin occurring in a number of our experiments in association with a fall in antithrombin, indicates a still greater absolute fall in the amount of prothrombin in the blood.

The following protocol illustrates the method by which our results were obtained. In this case the amount of prothrombin increased slightly while the amounts of antithrombin and fibrinogen decreased.

[10] Wright, J. H. and Kinnicutt, R.: Journal of the American Medical Association, 1911, lvi, 1457.

[11] Howell, W. H.: Archives of Internal Medicine, 1914, xiii, 76.

PROTOCOL—EXPERIMENT IV

Date, August 22, 1914.
Weight cat, 2 kilos. Urethane anaesthesia.
Anaesthesia begun, 11.40 a.m.
Operation begun, 12.03 p.m.

First Bleeding

Time, 12.11 p.m.
Amount, 23.5 cc.
Coagulation time (1) 14 minutes, 11 seconds.
(2) 13 minutes, 49 seconds.
Average, 14 minutes.

Second Bleeding

Time, 12.30 p.m.
Amount, 18 cc.
Coagulation time (1) 12 minutes, 12½ seconds.
(2) 14 minutes.
Average 13 minutes, 6¼ seconds.

Third Bleeding

Time, 12.50 p.m.
Amount, 18 cc.
Coagulation time (1) 12 minutes, 7 seconds.
(2) 12 minutes, 23 seconds.
Average 12 minutes, 15 seconds.
Death at 12.52 p.m.

ANTITHROMBIN DETERMINATION

Specimen 1

Thrombin drops	*Antithrombin drops*	*Time Interval minutes*	*Fibrinogen drops*	*Coagulation minutes*
3	1	15	10	15
4	1	15	10	10½
5	1	15	10	7¾
6	1	15	10	6
			Average,	9.81

Specimen 2

3	1	15	10	13½
4	1	15	10	9¼
5	1	15	10	6½
6	1	15	10	5¼
			Average,	8.62

Specimen 3

Thrombin drops	*Antithrombin drops*	*Time Internal minutes*	*Fibrinogen drops*	*Coagulation minutes*
3	1	15	10	6
4	1	15	10	$5\frac{1}{4}$
5	1	15	10	4
6	1	15	10	$3\frac{1}{4}$
			Average,	4.62

Control

Thrombin drops	*Fibrinogen drops*	*Coagulation minutes*
3	10	5
4	10	4

In making antithrombin determinations the selection of a definite end-point is somewhat difficult. The antithrombin delays or even prevents the formation of a solid clot. In our opinion the first definite appearance of coagulum in the clear solutions used is the safest end-point to employ, and we have used it throughout our determinations. In all of our experiments the average of the four antithrombin observations on each specimen has been used to represent the antithrombin factor in that specimen.

PROTHROMBIN DETERMINATION

Specimen 1

Ozalated Plasma drops	*0.5% $CaCl_2$ drops*	*Coagulation minutes*
5	2	5
5	3	5
5	4	$4\frac{1}{2}$
5	5	$4\frac{1}{2}$
5	6	$4\frac{1}{2}$

Tube containing optimum amount of $CaCl_2$ = $4\frac{1}{2}$ minutes.

Specimen 2

5	2	$3\frac{1}{2}$
5	3	$3\frac{1}{2}$
5	4	$3\frac{1}{2}$
5	5	3
5	6	3

Tube containing optimum amount of $CaCl_2$ = 3 minutes.

Specimen 3

Oxalathd Plasma drops	*0.5% $CaCl_2$ drops*	*Coagulation minutes*
5	2	$3\frac{1}{2}$
5	3	$3\frac{1}{4}$
5	4	3
5	5	$2\frac{1}{2}$
5	6	$2\frac{1}{2}$

Tube containing optimum amount of $CaCl_2$ = $2\frac{1}{2}$ minutes.

The end-point in each prothrombin reaction is reached at the moment when the tube can be inverted without dislodging the clot. Great care must be taken not to jar the tubes while the clots are forming. In all of our experiments the prothrombin factor in each specimen is represented by the lowest figure in the series—i.e., the time required for clotting in the tube containing the optimum amount of calcium.

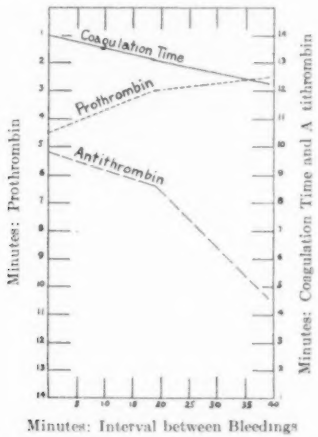

Fig. 1. Experiment IV.

FIBRINOGEN DETERMINATION

Specimen 1: 0.2137 gram in 100 cc. plasma.
Specimen 2: 0.1822 gram in 100 cc. plasma.
Specimen 3: 0.1350 gram in 100 cc. plasma.

Figure 1 (Exp. IV) represents the results of this experiment plotted graphically.

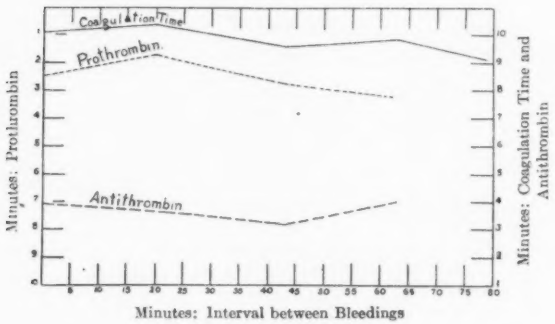

Fig. 2. Experiment IX.

Figure 2 (Exp. IX) represents the results of an experiment in which there was very little variation in the coagulation time, and a correspondingly small variation in the amount of antithrombin. The amount of fibrinogen increased slightly and then fell slightly, this experiment constituting one of the two exceptions to our observation of a steady fall in the amounts of fibrinogen. The amount of prothrombin rose slightly and then fell slightly, which in our experience was the most characteristic prothrombin behavior.

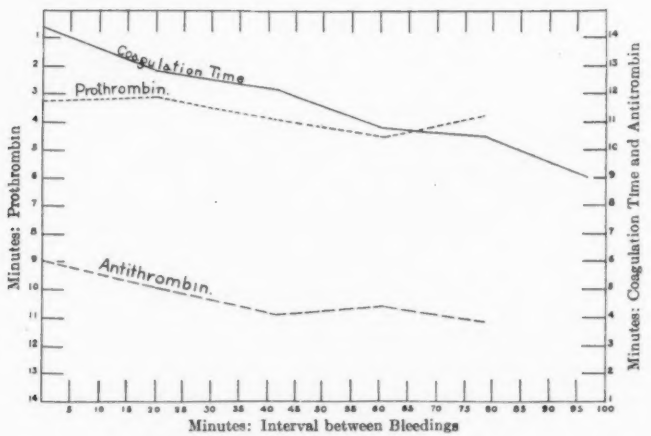

Fig. 3. Composite Curve, Cat.

Figure 3 is a composite curve embodying the coagulation times and the antithrombin and prothrombin determinations in our nine experiments on cats. The points to be noted are: (a) the fall in the amount of antithrombin corresponding to the decrease in coagulation time; and (b) a slight increase and then a slight fall in prothrombin. The final increase shown in the curve represents a single determination and is therefore unimportant. The fibrinogen determinations on cats are shown in Table 2, which contains a summary of the results of our entire series of experiments.

Discovering that at room temperature during a short period of contact with thrombin solution, the antithrombin in rabbits acts much more potently than does the antithrombin in cats, we determined to use the rabbit as a test animal.

The following protocol represents a typical rabbit experiment.

PROTOCOL—EXPERIMENT XV

Date, October 9, 1914.
Weight rabbit, 2.6 kilos. Urethane anaesthesia.
Anaesthesia begun, 10.35 a.m.

First Bleeding

Time, 11.22 a.m.
Amount, 13 cc.
Coagulation time (1) 18 minutes, 54 seconds.
(2) 18 minutes, 10 seconds.
Average 18 minutes, 32 seconds.

Second Bleeding

Time, 11.22½ a.m.
Amount, 13 cc.
Coagulation time (1) 18 minutes, 20 seconds.
(2) 18 minutes, 5 seconds.
Average 18 minutes, 12½ seconds.

Third Bleeding

Time, 11.47 a.m.
Amount, 12 cc.
Coagulation time (1) 15 minutes, 58 seconds.
(2) 15 minutes, 52 seconds.
Average 15 minutes, 55 seconds.

Fourth Bleeding

Time, 11.47½ a.m.
Amount, 12.5 cc.
Coagulation time (1) 14 minutes, 45 seconds.
(2) 16 minutes, 22 seconds.
Average 15 minutes, 33½ seconds.
Death at 11.55 a.m.

ANTITHROMBIN DETERMINATION

Specimen 1

Thrombin drops	*Antithrombin drops*	*Time Interval minutes*	*Fibrinogen drops*	*Coagulation minutes*
3	1	15	7	71¾
4	1	15	7	56
5	1	15	7	47¾
6	1	15	7	41¼
			Average,	54.18

Specimen 2

3	1	15	7	80½
4	1	15	7	62½
5	1	15	7	52
6	1	15	7	23½
			Average,	54.62

Specimen 3

3	1	15	7	57
4	1	15	7	46
5	1	15	7	33¾
6	1	15	7	15¼
			Average,	38

Specimen 4

3	1	15	7	64
4	1	15	7	47
5	1	15	7	33¼
6	1	15	7	23
			Average,	41.81

Control

Thrombin drops	*Fibrinogen drops*	*Coagulation minutes*
3	7	3½
4	7	2¼

PROTHROMBIN DETERMINATION

Specimen 1

Oxalated Plasma drops	*0.5% $CaCl_2$* drops	*Coagulation* minutes
5	2	$8\frac{1}{4}$
5	3	$6\frac{1}{2}$
5	4	$8\frac{1}{4}$
5	5	$7\frac{1}{2}$
5	6	$7\frac{3}{4}$

Tube containing optimum amount of $CaCl_2$ = $6\frac{1}{2}$ minutes.

Specimen 2

5	2	8
5	3	6
5	4	$7\frac{3}{4}$
5	5	$7\frac{1}{4}$
5	6	$9\frac{1}{4}$

Tube containing optimum amount of $CaCl_2$ = 6 minutes.

Specimen 3

5	2	$11\frac{1}{2}$
5	3	$8\frac{1}{4}$
5	4	$9\frac{1}{4}$
5	5	$9\frac{1}{4}$
5	6	$11\frac{1}{4}$

Tube containing optimum amount of $CaCl_2$ = $8\frac{1}{4}$ minutes.

Specimen 4

5	2	$9\frac{1}{4}$
5	3	$10\frac{1}{2}$
5	4	$10\frac{1}{2}$
5	5	10
5	6	$10\frac{1}{2}$

Tube containing optimum amount of $CaCl_2$ = $9\frac{1}{4}$ minutes.

FIBRINOGEN DETERMINATIONS

Specimens 1 and 2 (mixed): 0.3240 gram in 100 cc. plasma.

Specimens 3 and 4 (mixed): 0.2475 gram in 100 cc. plasma.

Figure 4 (Exp. XVI) shows a marked decrease in coagulation time; a marked fall in antithrombin; a fall in fibrinogen; first a decrease and then a final increase in prothrombin.

Figure 5 (Exp. X) shows a decrease in prothrombin after two bleedings and then a slight increase. The fibrinogen has in-

creased slightly (0.0168 gram) instead of falling. The coagulation time and the antithrombin have both decreased steadily.

Figure 6 is a composite figure embodying the coagulation times and the antithrombin and prothrombin determinations in

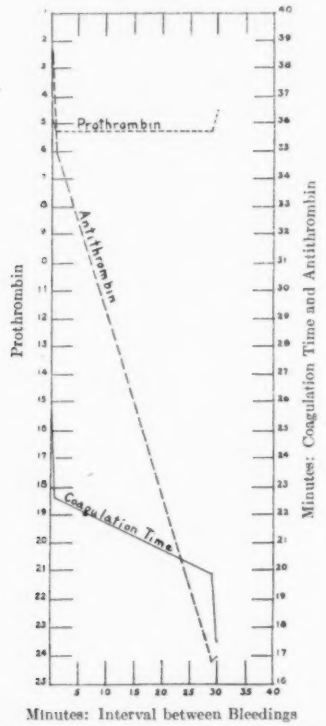

Fig. 4. Experiment XVI. Minutes 1 to 16 in the right hand column, being unnecessary in the figure, are omitted in order to save space.

our eight experiments on rabbits. It shows the same features which we have demonstrated in the composite curve of our experiments on cats: (a) a steady fall in antithrombin corresponding to a decrease in coagulation time; and (b) a slight increase, then a slight fall in the amount of prothrombin, and a final return to practically the original amount (two observations).

Our fibrinogen determinations on rabbits are shown in Table 2. The individual determinations vary less than those made upon cats but, with one exception, they show the same downward trend.

Thinking that possibly a marked change in the number of platelets after severe hemorrhage might be a factor to be con-

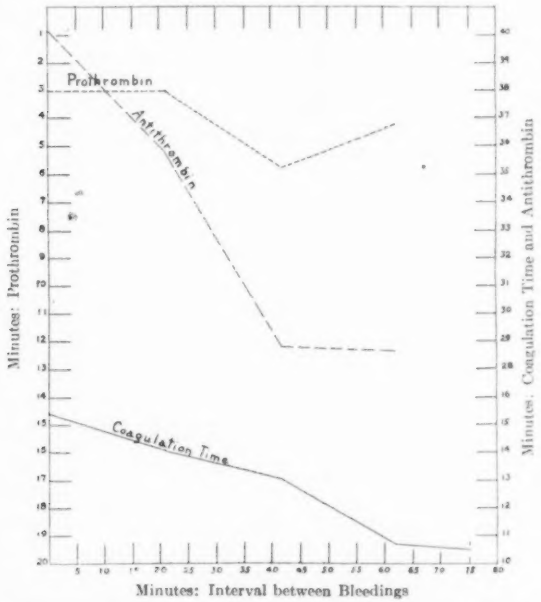

Fig. 5. Experiment X. Minutes 1 to 10 and 17 to 28 in the right hand column, being unnecessary in the figure, are omitted in order to save space.

sidered, we made platelet counts in five experiments before the first hemorrhage and then after each bleeding, but found practically no change in their number.

A comparison of the average results of our experiments on cats and those on rabbits is of interest. The behavior of the prothrombin in the two series of experiments was in general the same—a slight initial increase and then a slight decrease in

amount. In both series there was a steady fall in fibrinogen: in the cats an average decrease of 0.0762 gram per 100 cc. of plasma after three bleedings: in the rabbits an average decrease of 0.0770 gram after three bleedings and 0.0811 gram after four bleedings. The following table gives a comparison of the per-

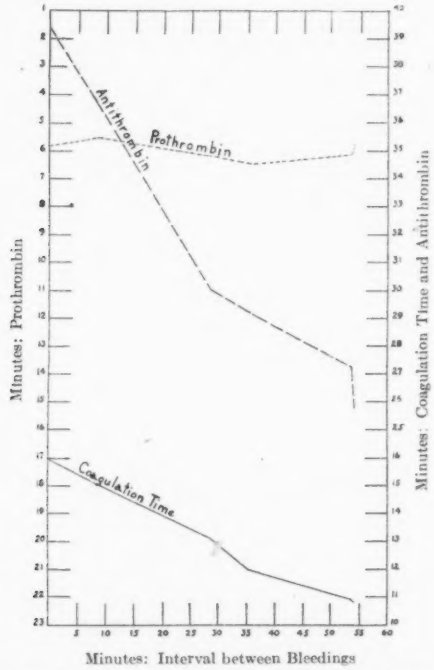

Fig. 6. Composite Curve, Rabbit. Minutes 1 to 10 and 17 to 25 in the right hand column, being unnecessary in the figure, are omitted in order to save space.

centages of the average decrease in coagulation times and in the amounts of antithrombin following each hemorrhage in the two series of experiments.

TABLE 1

	CAT		RABBIT	
	Percentage decrease in coagulation time	Percentage decrease in antithrombin	Percentage decrease in coagulation time	Percentage decrease in antithrombin
	per cent	*per cent*	*per cent*	*per cent*
2d specimen	11.1	15.1	5.4	6.7
3d specimen	15.4	30.9	17.8	23.6
4th specimen	25.0	26.4	24.8	25.9
5th specimen	27.0	34.6	31.6	30.8
6th specimen	37.4	No specimen obtained	32.2	34.7

Table 2 gives a summary of the results obtained in our entire series of experiments.

DISCUSSION AND CONCLUSIONS

Coagulation Time. Our experiments, in accord with the observations of other investigators, show that rapid progressive hemorrhage causes a decrease in coagulation time. An occasional animal proves an exception to this rule, and shows practically no change in its rate of clotting no matter how severe the hemorrhage (e.g., Exps. II and XI in Table 2), but the majority of the animals show a gradual steady fall in the rate of coagulation as the hemorrhage proceeds.

Antithrombin. In our series of experiments, the decrease in coagulation time is accompanied by a decrease in the amount of antithrombin. Whether or not this change is the result of a simple dilution of the blood by an influx of fluid from the tissues, or whether it is due to a decrease in the amount of antithrombin formed, we are unable to say. In either case the fact of diminution remains. Gray and Lunt[12] have shown that there is no decrease in coagulation time after hemorrhage in an anterior animal (i.e. an animal in which the aorta and vena cava are ligated just above the diaphragm). This observation may be easily reconciled with our experiments. If the decrease in antithrombin

[12] Gray and Lunt: Loc. Cit. (1).

TABLE 2

Cat

Specimens	COAGULATION TIME												INTERVAL BETWEEN BLEEDINGS					ANTITHROMBIN						PROTHROMBIN						FIBRINOGEN			
	1		2		3		4		5		6		1–2	2–3	3–4	4–5	5–6	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4
	min.	sec.	min.	sec.	min.	sec.	min.	sec.	min.	sec.	min.	sec.	min.	min.	min.	min.	min.	min.	min.	min.	min.	min.	min.	min.	min.	min.	min.	min.	min.	gm.	gm.	gm.	gm.
Exp. I	11	38	10	36½	9	45	7	44					14	30	19			7.56	6.00	5.50	5.00			2	2	4	4½			Determination			Lost
Exp. II	10	35¾	10	11	10	0	9	¾					16	15	15			Determination Lost						2½	2½	2	4½			0.4758	0.4016	0.3408	
Exp. III	19	0	12	27	14	54½	15	7					28	16	19			6.75	6.50	4.87	4.87			3½	3	3¾	5			0.0675	0.0652	0.0644	
Exp. IV	14	0	13	6¼	12	15							19	20				9.81	8.62	4.62				4½	3	2½				0.2137	0.1822	0.1350	
Exp. V	15	27½	11	16	11	31½	11	35					20	20	20			Determination Lost						2½	4¼	3½	5			0.3611	0.3116	0.2430	
Exp. VI	15	16½	12	59½	10	47							20	29				4.50	3.68	3.62				5	3¼	3½				0.2216	0.1049	0.0974	
Exp. VII	19	34½	20	44½	18	12½							23	23				4.12	3.31	3.18				3	5	8½				0.5342	0.5062	0.4134	
Exp. VIII	14	0	13	33	12	32	11	35	11	54½	9	1	20	21	20	20	18	5.43	4.00	4.12	3.81	3.93		4	3½	4½	5	3¾		0.0843	0.0843	0.0562	0.0481
Exp. IX	10	4	10	20	9	35½	9	49½	9	6			20	23	20	16		3.93	3.62	3.18	4.00			2½	1¾	2¾	3¼			0.2373	0.2666	0.2361	
Average in minutes	14.4		12.8		12.17		10.8		10.5		9.01		20	21.88	18.83	18	18	6.01	5.10	4.15	4.42	3.93		3.27	3.13	3.86	4.54	3.75		0.2744	0.2403	0.1982	0.0481

Rabbit

Exp. X	15	26	14	4½	13	2½	10	45½	10	31			21	21	20	13		40.18	35.81	28.80	28.68			3	3	5¼	4¼			0.2925	0.2947	0.3093	
Exp. XI	15	5½	14	35	15	17							20	21				23.06	24.50	20.93				2¾	2½	3¾				0.3240	0.2812	0.2328	
Exp. XII	15	56	13	34	9	3	12	41	12	28	12	25	½	21½	½	21	½	41.50	39.68	32.43	33.56	30.12	25.56	5¼	6½	8	6	6½	6	Determination not made			
Exp. XIII	12	16	11	54	8	8½	8	55	9	48	9	15	½	20½	½	20½	½	36.06	36.37	28.68	23.81	24.43	25.87	8½	6	5¼	6¼	5¾	5½	Determination not made			
Exp. XIV	9	37	11	17	9	39	9	21					21	½	24			38.12	31.43	39.06	23.12			9½	8¾	4½	4¾			Determination not made			
Exp. XV	18	32	18	12½	15	55	15	33½					½	24½	½			54.18	54.62	38.00	41.81			6½	6	8¾	9½			0.3240	0.3240	0.2475	0.2475
Exp. XVI	25	55	22	38	19	53	17	30½					½	28½	1			38.62	35.06	16.75	16.87			4¾	5¼	5¼	4½			0.2621	0.2621	0.1968	0.1968
Exp. XVII	15	4	14	40	14	4	9	15					2	24½	1½			43.75	36.56	36.18	36.68			6¼	6½	7¾	9¾			0.5062	0.5062	0.3375	0.3375
Average in minutes	15.98		15.11		13.12		12.00		10.92		10.83		8.25	20.25	6.85	18.16	0.5	39.43	36.75	30.10	29.21	27.27	25.71	5.81	5.56	6.18	6.46	6.12	5.75	0.3417	0.3336	0.2647	0.2606

which we have observed is due to a decrease in the amount formed, we may assume that antithrombin is formed in one of the abdominal organs. In support of such an assumption there is considerable evidence to show that the liver is very active in antithrombin production.

If the decrease in antithrombin is a matter of dilution, we know that the major portion of the fluid entering the blood after hemorrhage comes from the abdominal region. This fluid which enters the blood after hemorrhage is, in our opinion, lymph, not tissue juice. If it could be shown that tissue juice—the fluid obtained from wounded tissues—entered the blood after hemorrhage, we could readily explain our diminished amount of antithrombin by assuming that a large portion of it was neutralized or fixed by the thromboplastin in the tissue juice. In this way the amount of free antithrombin in the blood would be diminished, and our tests for it would show this reduction. But lymph, on the contrary, is not rich in thromboplastin. Howell[13] has shown very recently that lymph contains prothrombin and antithrombin in the same concentration as blood plasma, but much less thromboplastin. There is, therefore, a relative excess of antithrombin in lymph which explains its long coagulation time. Addition of thromboplastic material (tissue extract or kephalin solutions) causes lymph to clot promptly and firmly. In view of these facts it would seem that an influx of lymph into the blood after hemorrhage would bring with it a relative excess of antithrombin, which would increase the coagulation time of the blood instead of decreasing it. It is perhaps more reasonable to suppose that the decrease in antithrombin which we have demonstrated is the result of diminished antithrombin production.

Prothrombin. The behavior of the prothrombin in our experiments varied in different animals: occasionally it increased steadily in amount, occasionally it decreased steadily, but in the majority of cases it first increased slightly and then decreased slightly. The only conclusion in regard to the prothrom-

[13] Howell, W. H.: American Journal of Physiology, 1914, xxxv, 483.

bin which we feel warranted in making is that the prothrombin changes in our series of experiments do not offer any explanation for the decrease in coagulation time which occurred.

Fibrinogen. The fibrinogen in our experiments, as estimated by the heat coagulation method, gave marked variations as to the amount present in different animals, a fact in accord with Whipple's observations,[14] but remarkably uniform results as to the effect of hemorrhage on the fibrinogen content of the blood of individual animals.

As time has passed we have felt more and more certain that this method of estimating fibrinogen is open to question. In Table 2, it may be noted that while one cat shows a normal content of 0.0675 gram of fibrinogen per 100 cc. of plasma, another cat shows a normal content of 0.5342 gram. These figures give the maximal variation, 0.4667 gram, which we found between two different animals. Whipple's maximal variation in dogs was 0.6686 gram per 100 cc. of plasma, but he never obtained in normal animals the very low figures of 0.0675 gram and 0.0843 gram which we encountered twice in this series of experiments, and which we have found a number of times in some experiments on rabbits not yet reported. None of these animals possessing an apparently very low fibrinogen content showed any bleeding tendencies but seemed normal and healthy in every way. We have taken every precaution to assure ourselves that there has been no technical error in the application of the method, and we are convinced that there is enough variation in the reactivity of fibrinogen to heat to render the heat coagulation method of determining this substance of somewhat questionable accuracy.

Our data in regard to fibrinogen is as follows. With the exception of two cases (Exps. IX and X) all of our determinations showed a steady decrease in the amount of fibrinogen following each hemorrhage. The average fall from the normal in all cases of specimens obtained at the third bleeding was 0.0766 gram per 100 cc. plasma.

[14] Whipple, G. H.: American Journal of Physiology, 1914, xxxiii, 50.

Dreyer[15] has shown that the fibrinogen content of the blood increases after hemorrhage provided the interval between bleedings is a period of twenty-four hours. It is possible that in our experiments the apparent reducing effect of rapid progressive hemorrhage upon the fibrinogen content of the blood, is in reality a false one. It may be that instead of an actual fall in the amount of the fibrinogen, there occurs some alteration in the fibrinogen itself which diminishes its responsiveness to the heat coagulation test. On the other hand we must remember that Nasse, Brücke, and von den Velden have all reported an immediate diminution in the fibrin content of the blood following severe hemorrhage, and at the same time an increase in the speed of coagulation.

There remains to be considered the question as to whether or not the results which we have obtained in our analyses of the factors of coagulation after hemorrhage are sufficient to explain the decrease in coagulation time which occurred, but this question unfortunately must remain unanswered. A decrease in antithrombin will of course favor more rapid clotting, but whether or not the decrease which we have shown is sufficient to compensate for a certain amount of diminution in prothrombin and for a possible diminution in fibrinogen also, we are unable to say. The fact that there was very little change in antithrombin in two of our experiments in which the coagulation time was practically constant seems suggestive in this connection.

We believe that the chief interest in these experiments lies in our demonstrations of a variation in antithrombin. Except for the experiments of Davis[16] showing the production of an antithrombin wave as the result of thrombin injections and the work of various investigators on the increase in antithrombin as the result of peptone injections, all previous work on the antithrombin in the blood has indicated that this substance is remarkably constant. Thrombin and peptone injections produce

[15] Dreyer, G. P.: Studies from the Biological Laboratory of the Johns Hopkins University, 1893, v, 77.

[16] Davis, D.: American Journal of Physiology, 1911, xxix, 160.

an increase in the amount of antithrombin. Our experiments are the first instance in which there has been reported a positive decrease in the antithrombin content of the blood.

SUMMARY

1. Rapid progressive hemorrhage causes a progressive decrease in coagulation time. An occasional animal proves an exception to this rule and shows no change in its rate of clotting, no matter how severe the hemorrhage.

2. Antithrombin decreases in amount when the coagulation time decreases and remains practically constant when the coagulation time is unchanged.

3. Prothrombin tends first to increase slightly in amount and then to decrease slightly.

4. Fibrinogen, estimated by the heat coagulation method, decreases as hemorrhage progresses.

5. Platelets counts do not vary with rapid progressive hemorrhage.

We wish to thank Dr. W. B. Cannon for his interest and advice.

THE VASOMOTOR NERVES OF THE PORTAL VEIN

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The experiments of Mall[1] pertaining to the influence of the portal system upon the distribution of the blood, have been cited repeatedly in proof of the contention that the portal vein is controlled by vasomotor nerves. The observation which is generally regarded as adequate to fully substantiate this conclusion, consists in the fact that high occlusion of the abdominal aorta fails to destroy the rise in the carotid blood pressure which usually accompanies splanchnic stimulation. Mall states that "Wenn sich die Aorta so verstopfen lässt, dass den Arterienästen unterhalb des Zwerchfells kein Blut mehr zufliesst, und wenn dann die Reizung des Nervus splanchnicus den Druck in der Carotis noch steigert, so ist damit streng bewiessen, dass nur die aus dem Portalsystem durch das Herz in das Aortenwerk eingeführten Blutmassen den Druck erhöht haben."

It is the purpose of the present discussion to show that this "proof" is open to several serious objections. While, no doubt, an augmentation in the carotid blood pressure can be obtained under the conditions here briefly outlined, the conclusion based upon it, that the portal vein is innervated by veno-motor nerves, must be regarded as not having been substantiated.

Mall states that no blood can reach the arteries below the diaphragm, if the aorta is occluded by a ligature at a point directly below the origin of the arteria subclavia sinistra. This ligation having been effected, the crural artery will always be found free from blood, but such a result cannot be attained if the aorta is obstructed immediately above the diaphragm.

[1] Archiv für Anat. und Physiologie, 1892, 409.

The statement is also made that the occlusion of this blood vessel is compensated for in the course of time. Quite similarly, stimulation of the splanchnic nerves results in an augmentation of the general blood pressure, but the rise is more marked if the posterior part of the body is first rendered "bloodless." Thus, values of 200 mm. Hg. are seldom reached with the aorta intact, while the compression of this blood vessel enables the pressure to rise to points considerably above this level. In accordance with Mall this secondary rise finds its origin in a rapid influx of blood from the portal vein occasioned by the constriction of this blood vessel.

Contrary to Mall it has been demonstrated by Vehlich[2] that a certain amount of blood will find its way into the crural artery of the dog even after the aorta has been ligated below the subclavian artery. A similar result has been obtained by Hering[3] who states that this procedure renders the arteries of the abdominal cavity of rabbits only partially bloodless, i.e., "beim Aufschneiden fliessen immer noch ein bis zwei Tropfen Blut ab."

The conclusion to be drawn from these experiments is that the compression of the aorta at the point indicated does not lead to a confinement of the blood in anterior channels, but rather favors its escape from them by opening up a very effective system of anastomosing blood vessels. By dividing the body transversely Vehlich has shown that these connecting blood vessels are located within the vertebral column. It must be admitted, however, that the establishment of this fact by physiological means has not augmented our knowledge materially, because this anastomosis seems to have been clearly recognized many years before the time of publication of the article of Vehlich and even before the appearance of the paper of Mall. Thus, Ellenberger and Baum[4] state that the arteria spinalis anterior passes to the posterior end of the cord by making use of the sulcus longitudinalis anterior as its highway. This blood vessel receives connecting branches from the intercostal, lumbar and sacral

[2] Pflüger's Archiv, xcv, 1903, 264.
[3] Pflüger's Archiv, lxxii, 1898.
[4] Anatomie des Hundes, Berlin, 1891.

arteries. Moreover, Heidenhain[5] made the observation that occlusion of the aorta does not always provoke a fall in the crural blood pressure to zero and concluded, therefore, that an arterial communication is present somewhere in the domain of the spinal column.[6]

The existence of an anastomosis of this kind is also made very probable by the experiments of Basch and Oser.[7] Having ligated the aorta above the diaphragm, these authors injected nicotine into the current of the carotid artery. Very pronounced contractions of the intestine resulted in consequence of this procedure which, however, were interpreted as proving that this drug possesses a central action. Under quite similar experimental conditions Pal[8] employed morphine to test the effect of this agent upon the centers of the splanchnic nerves. Luchsinger[9] believed that the action of pilocarpin in causing a secretion of sweat upon the paws of the cat's foot, is entirely central, because this effect could also be obtained after ligation of the abdominal aorta.

This deduction, however, does not seem to be justified, because Robillard[10] has shown that the injection of pilocarpin does not produce a secretion of sweat if the tibial nerve is made to form the only connection between the foot and the remaining portion of the posterior extremity. A true central stimulation, as may be had, for example, by an accumulation of carbon dioxide, will, however, readily produce a secretion of sweat at this time. Sodium iodide injected into the carotid artery or jugular vein of dogs, could easily be shown to be present in the blood of the crural artery after ligation of the aorta. It could also be detected after these animals had been divided transversely in such a way that the spinal column formed the only connecting bridge between the anterior and posterior segments of their bodies. This proof of Vehlich has been greatly amplified by him. For

[5] Pflüger's Archiv, xlix, 226.
[6] Compare: Mares, Pflüger's Archiv, xlvii, 1903, 567.
[7] Wiener: Med. Jahrbücher, 1872.
[8] Wiener: Med. Presse, 1900, No. 45.
[9] Pflüger's Archiv, xv, 1877, 482.
[10] See Biedel, Wiener Med. Presse, 1899.

example, he has been able to incite rises in the crural blood pressure by injecting adrenalin into the vascular circuits of the fore part of the body and has succeeded in recovering from this blood vessel coloring material that had been added to the carotid blood stream.

These experiments, as well as others that might still be mentioned, prove conclusively that the ligation of the thoracic aorta, as practised by Pal and Mall, does not lead to a cessation of the circulation through the channels located below the diaphragm. Hence, all conclusions which are based upon an assumption contrary to this fact, cannot be regarded as being in agreement with well established anatomical and physiological data.

The second discrepancy pertains to the size and location of the vascular field which is controlled by the splanchnic nerves. The conclusion of Mall relative to the motor power of the portal vein, is based upon the phenomenon that stimulation of the greater splanchnic nerves induces rises in the carotid blood pressure even after the thoracic aorta has been ligated. The claim is made that the constriction of this bloodvessel causes a transfer of portal blood into the arterial channels sufficient to produce this change. It must be evident that if the augmentation in the carotid pressure is to be regarded as an indication of an active reduction in the size of the portal bloodbed, the vascular field governed by the splanchnic nerves, must be carefully restricted to this venous channel. The question whether Mall has actually succeeded in narrowing the area experimented upon in a satisfactory manner must be answered negatively.

Based largely upon the presentation of Swan,[11] it is stated by Mall that each splanchnic nerve arises by three separate strings, namely, as the splanchnicus major, minor and tertius. Dense networks of fibres appear in the vicinity of the adrenal bodies, from which others continue onward to aid in the formation of the superior mesenteric and coeliac masses of the solar plexus. To quote further: "Ausser den Zweigen zum Darm kanal und seinen

[11] Comparative anatomy of the nervous system, London, 1864.

Drüsen sendet das Ganglion noch zuweilen solche zur Niere." Obviously, the picture which is drawn here regarding the distribution of the splanchnicus is very indefinite, and is much less accurate than the description given upon page 569 of Ellenberger and Baum's *Anatomie des Hundes*. As this work has been cited by Mall, its contents pertaining to the sympathetic innervation of the abdominal parts, must or should have come to his attention.

While still incomplete, the description of Ellenberger and Baum is not at all inconclusive or evasive regarding the sympathetic innervation of the kidneys. Contrary to the preceding quotation, it is stated here very clearly that the "rami renales kommen aus der caudalen Seite des Geflechtes—und bilden im Verein mit Zweigen vom Ganglion mesentericum superius um die Nierenarterie den Plexus renalis; die Fäden desselben umspinnen die Nierenarterien und dringen in den Nierenhilus ein." These authors even mention the fact that the splanchnic nerves supply the supra-renal bodies; and possess other intra-abdominal ramifications.

It must be conceded, therefore, that the nerves in question control a vascular field which is very much larger than that drained by the portal vein. Hence, when the stimulation of the splanchnic nerves incites an augmentation in the carotid blood pressure, this rise cannot be said to have been caused solely by constrictive reactions in the portal vein. In accordance with the evidence just submitted, they may indeed be referred to motor activities in several other circuits possessing a sympathetic nerve supply. The possible contention that, if compared with the portal blood flow, these extra-portal blood streams become of negligible importance, cannot justly be offered in support of the conclusions of Mall, because a brief comparison of the blood supply of the kidneys with the portal bloodflow will show the opposite to be true. In accordance with the calibrations furnished by Burton-Opitz,[12] a liver weighing 500 grams receives about 7.0 cc. of blood in a second or 420 cc. in a minute. Of

[12] Quarterly Journal of Experimental Physiology, v, 1912, 189.

this total amount nearly three-fourths are gathered from the portal organs. A liver of this weight we may expect to find in a dog weighing about 15 kg. and, as the total quantity of blood present in an animal may be roughly calculated at one-thirteenth of the body weight, the entire mass of blood must traverse this organ once in every three minutes.

When reference is had solely to the total blood supply of organs, the liver no doubt ranks first among the structures of the body, but if especial emphasis is placed upon the amount of blood that is allotted to each 100 grams of substance in a unit of time, this organ cannot be said to be as vascular as the kidney. Upon this basis, the combined arterial and venous supply of the liver amounts to only 1.40 cc. in a second or to 84 cc. in a minute, while the venous stream alone possesses a value of 59 cc. in a minute. Opposed to this figure, we have a minute-volume for the renal vein of 151 cc. per 100 gram of kidney-substance.[13] Clearly, therefore, the kidney is an organ that must be reckoned with. It is compact and, while it does not contain a considerable quantity of "residual" blood, it is capable of taking up large amounts of blood by simply permitting the latter to flow through its channels without much hindrance. Thus, we know that the renal vein frequently discharges a quality of blood which is markedly lighter than that contained in the vena cava; its color, in fact, often closely approaches that of the arterial blood. Excepting the thyroid body, the kidney is the most vascular structure in the body and naturally, the value given previously gains much in importance, if it is doubled to represent the amount of blood discharged by the two organs.[14]

It has been shown by Burton-Opitz and Lucas that the shutting off of the arterial supply from one of these organs suffices to cause an augmentation in the general blood pressure. Similarly, it has been proven by them that this result may also be attained by excitation of the greater splanchnic nerves. Hence, the quan-

[13] See: Burton-Opitz and Lucas, Pflüger's Archiv, cxxiii, 1908, 156.

[14] In a dog weighing about 16 kg. each kidney discharges 1.64 cc. of blood in a second. The weight of each organ amounts to 65 grams; hence, each 100 grams of substance receive 151 cc. of blood in a minute.

tity of "residual" blood which is discharged under these conditions by the constricting renal bloodvessels, must indeed be considerable. If now it is taken into account that Mall has also disregarded the haemodynamical influence of other extra-portal structures; for example, that of the supra-renal bodies, it becomes quite evident that the rises in the carotid blood pressure obtained under the conditions previously outlined, cannot be referred solely to constrictive reactions in the portal vein.

In order to amplify his results Mall has also resorted to excitation of the splanchnic nerves after temporary occlusion of the portal vein and vena cava below the diaphragm. The carotid pressure was observed to decline subsequent to the ligation, but was seen to rise during the period of stimulation. In accordance with this result the statement is made that the splanchnic nerves undoubtedly possess the power of removing the blood from the liver. The stimulations were confined at first to the different roots of the splanchnic nerve, but a record was also made of the excitation of the peripheral end of the network of fibres accompanying the hepatic artery.

Whether the conclusion just quoted has been satisfactorily proven by the tests just briefly outlined, must remain doubtful, because no data are given regarding the exact location of the ligatures. The anastomosis between the different portal tributaries, as well as between this system and more central channels, is very complete. We have seen previously that one of the essential preliminary procedures, namely, the obstruction of the thoracic aorta, has failed in its purpose on account of the presence of anastomosing arterial channels. It has been proven that a cessation of the arterial influx cannot be accomplished in this way.

Concurrently, no reason seems to be at hand to assume that the object which Mall hoped to attain by obstructing the inferior cava, has actually been attained. In fact, it is admitted in the paragraphs now under discussion that the vena portae is connected with the heart by more than one channel. It is stated here that the path of least resistance leads through the liver, and the one of greatest resistance through the vena azygos. Mall also

shows that a communication exists between the mesenteric veins and the vena azygos.

Resort was also taken to the ligation of the inferior cava above the diaphragm and hence, presumably, centrally to the orifice of the hepatic vein. When this blood-vessel was obstructed in addition to the thoracic aorta, the excitation of the splanchnic nerves failed to induce the characteristic augmenting effect in the carotid blood pressure. In fact, this procedure was invariably followed by a decline in the general blood pressure. In illustration of this result I quote the figures of a part of experiment II. To begin with, the carotid pressure amounted to 134 mm. Hg. Measured at intervals of 10 seconds, the ligature of the cava above the diaphragm induced the following values: 104, 80, 47, 36, 22, and 14 mm. Hg.

While the absence of an augmentation in the carotid blood pressure fails in this case to strengthen the assumption that the portal vein possesses constrictive powers, it proves very suggestive in another way. To be sure, any confinement of the blood in anterior vascular channels produces at first a decided rise in the carotid blood pressure which, however, is compensated for in a measure within a reasonable time thereafter. It appears to me that the fall in blood pressure of 120 mm. Hg obtained within a period of 60 seconds, does not point so much towards a compensatory approximation of the anterior vascular channels as towards a rather free leakage of blood into the supposedly tight vascular compartments posterior to the ligatures. This result might indeed, have suggested to Mall that the occlusion of the thoracic aorta does not prevent the blood from escaping into posterior bloodvessels.

In referring to the description of the splanchnic nerves by Ellenberger and Baum, the statement is made by Mall that: "In letzterem Werke werden abweichend von meinen Befunden statt eines drei Plexus supra-renales beschrieben." I quote from page 569 of this work as follows: "Nahe dem dorsalen Rande derselben (Nebenniere) nimmt der N. splanchnicus ein Ganglion in seine Bahn auf, von dem ein stärkerer oder zwei schwächere Zweige nach dem Ganglion coeliacum, das zur Seite

der A. coeliaca liegt, abgehen, während ein anderer Zweig sich mehr caudal wendet und noch ein bis zwei kleinere Ganglien aufnimmt. Dabei verbindet sich dieser Zweig mit den drei splanchnici minores und bildet mit ihnen gemeinsam das an der medialen Fläche der Nebenniere gelegene Nebennierengeflecht (Plexus supra-renalis). Aus demselben und zwar wesentlich aus seinen Ganglien gehen, etc." I venture to believe that a scrutiny of this description will show that Ellenberger and Baum should not be charged with having recognized three plexus supra-renales. The fact that they have not fallen into so obvious an error is shown especially well towards the end of this description, where they consistently refer to this plexus by the singular term.

I would also call attention to another misconception. If it is granted that stimulation of the peripheral end of the hepatic plexus leads to the removal of a certain quantity of blood from the liver even after the vena portae has been ligated, the deduction might seem justifiable that this bloodvessel possesses motor activity. Such a conclusion, I venture to believe, is not warranted upon the basis of Mall's experiments, because the liver derives its blood from two sources. Obviously, therefore, the preceding deduction can be accepted as proven only under the condition that the arterial supply has been prevented from reaching this organ. In the first place, considerable histological evidence is at hand to show that the arterial and portal terminals communicate with one another in the liver.[15] Secondly, it has been established by Burton-Opitz[16] that the occlusion of the portal channel at the hilus does not lessen the blood supply of this organ in a corresponding measure, but whenever such a procedure is attempted, an increase in the arterial influx results which in part compensates for the quantity of portal blood lost.

My objections to the conclusions now under discussion are only technical, however, because I myself have shown that the intra-hepatic bloodvessels are equipped with motor mechanisms. One of these is situated in the terminals of the hepatic artery

[15] See Gad, Dissertation, Berlin, 1873. Also Köllicker's Gewebelehre.

[16] Quarterly Journal of Experimental Physiology, ii, 1911, 93.

and the other in the radicles of the portal vein.[17] This result has been arrived at (a) for the arterial channels under exclusion of the portal blood, and (b) for the venous tubules under exclusion of the arterial influx. Again, I have sought to establish the existence of these mechanisms in each case separately by calibration of the arterial and portal blood streams before and during the stimulation of the hepatic nerve fibres, as well as before and after the administration of varying quantities of adrenalin.

In this connection mention must also be made of the fact that the term "portal vein," as formerly used, no longer conveys a clear impression regarding the exact seat of the vasomotor reactions. Reference may be had, on the one hand, to the truncal portion of the vein and, on the other, either to its terminals in the liver or to its radicles in the different organs forming the portal system. While we know that the arterial entrance to the portal vein is well guarded peripherally by very efficient motor mechanisms located within the domain of the different "portal" organs, it cannot be said that the existence of such a mechanism in the truncal segment of this channel has been definitely established. I believe that in the absence of more specific statements, Mall's references to the portal vein are usually thought to apply to the trunk of this bloodvessel.

I venture to believe that the statements of Mall quoted in this paper, have been disproven in a satisfactory manner without that a further discussion of minor errors becomes necessary. As has been emphasized previously, my principal objections against the conclusions of Mall are (a) the improper limitation of the vascular fields experimented upon (b) the only partial degree of anaemia which ligation of the thoracic aorta produces, and (c) the imperfect localization of the reactions for which the intra and extrahepatic anastomosis of the blood-vessels are responsible.

[17] Quarterly Journal of Experimental Physiology, vii, 1913, 57.

THE INFLUENCE OF PUPILLARY DIAMETER ON VISUAL ACUITY

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INTRODUCTORY

One of the commonly accepted functions of the pupil of the eye is that of compensating its dioptric defects. Owing to the chromatic and spherical aberration of the refractive system of the eye the light from a bright point of an outer object does not come accurately to a focus upon the retina, but forms there a small diffusion circle of light, which if large enough has blurred vision as its effect. Astigmatism adds to this effect, and if irregular introduces additional irregularities, causing still further imperfectness of the image. The pupil by reducing the size of the light-pencil, reduces also the size of the diffusion-circle and other irregularities, gives a clearer image and consequently better vision. The present work was undertaken to estimate the importance of this function of the pupil and further to find by experiment how far the eye is actually subject to that which physicists designate as the resolving power of its optical system. The image of a bright point, such as a star, when formed by the object-glass of a telescope, and looked at through a highly magnifying eye-piece, is seen not to be a mere point of light, but a central point of light in a concentric series of dark and bright annuli, each bright ring being of greater intensity than those surrounding it, and the central point being the brightest part of the figure. This pattern is due to diffraction and interference of the light waves passing through the lens-aperture, and is not to be confounded with the diffusion circle previously spoken of, for it is something wholly different. A detailed explanation of its origin can be found in the text-books on physical optics (1), (2), (3).

It is sufficient to state here that it has been empirically found that the eye is just able to differentiate two such images when the central point of one falls in the darkest part of the inmost dark ring of the other, independently of the magnification introduced by the eye-piece. The condition of this is expressed by the formula.

$$\theta = 1.22 \frac{\lambda}{D}$$

Where θ = the angular separation of the two bright points subtended at the optical center of the image-forming system $\left(\text{expressed as } \frac{\text{arc}}{\text{radius}}\right)$,

λ = the wave-length of the light concerned,

and D = the diameter of the lens-aperture (these last two being expressed in equal units).

Inspection of the formula shows that the larger D is, the smaller is θ. That is, the resolving power is proportionately greater, the larger the aperture. This is contrary in sense to the usually accepted view as to the effect of the pupil of the eye, and presupposes a lens system free from defects. From this it would at once appear possible, by using a sufficiently small diaphragm before the eye, to so reduce the effectiveness of its optical imperfections, and at the same time decrease its resolving power as explained above, that the eye would be subject chiefly to the latter, and that under certain conditions at least a smaller aperture would actually give lower visual acuity than a larger one. The present work shows that this effect can be obtained, and just how far it is effective.

HISTORICAL

The writer has found in the literature only two systematic investigations on the effect of pupillary width upon visual acuity. Uhthoff (4) in 1890 studied this question as a preliminary one to determine the optimal size of an artificial pupil to use in the investigation of visual acuity in spectral light of different wavelengths and intensities. He used diaphragms of various sizes

(1.06 to 3.02 mm. diameter) and altered the slit-width of his spectrometer system inversely as the area of the diaphragm used, so as to get equal luminous flux into the eye in all cases. He found that under high illumination a certain diaphragm of 2.06 mm. diameter gave the best vision, the next larger (3.02 mm.) and next smaller (1.55 mm.) giving lower values. In other sets of observations at lower brightness (less energy admitted to the eye) smaller apertures gave optimal results. Vision of course was lower throughout these sets. The results were given for wavelengths 505 $\mu\mu$ and 605 $\mu\mu$, in both cases showing the facts just stated. He attributed this effect to diffraction, noting that with a 1 mm. aperture the limbs of the test-object appeared indistinct and broader as the limit of vision was reached, whereas with the larger diaphragms they continued to be sharply outlined up to the point of unrecognizability. He concluded that the diaphragm of 2.06 mm. diameter was the best one to use, as above that the optical irregularities of the eyes were in evidence.

Hummelsheim (5) in 1898 used homatropin and pilocarpin to vary the size of the pupil, and determined visual acuity by means of the Snellen illiterate character **E** under daylight illuminations varying from 1 to 200 mk.[1] The illumination was controlled by window shades and measured with a Weber photometer. His results showed visual acuity with the contracted pupil to be higher and with the dilated pupil lower than with the normal pupil, the differences showing a tendency to disappear at low illuminations, under which conditions vision dropped to lower values in all cases. The smallest pupil worked with in his two observers was 1.5 mm. in the case of both eyes of the one and 2.25 mm. in the other.

[1] Meter-Kerzen. The candle referred to is the Hefner unit; approximately 0.9 international candle.

THE PRESENT WORK

Apparatus

The test object used is one which has already been described (6) [see also (7) (8)] and permits of continuous variation in the width of a set of parallel bands without altering any other factor in the stimulus. The average brightness of the test field is equal for all widths of the bands. The observer, by means of a small hand-wheel, pulleys and a cord, is enabled to adjust the object while observing it, and instructed to keep it just at the limit of visibility, that is to make the lines gradually smaller while they are visible, and to increase their width while invisible. The movements of the test-object thus brought about are recorded on a kymograph drum by means of a lever and writing point. At the beginning or at the close of the experiment the abscissa lines are drawn in by setting the test-object successively at even millimeters and giving the drum one revolution. To get the mean setting of the instrument for any period it is necessary to scale the ordinate at each time-mark (every 5 seconds of the period) and compute the setting of the instrument corresponding to the mean ordinate.

The changes in the eye-aperture are brought about by using small diaphragms of blackened brass, as close as possible to the observing eye. The distance from the eye to the plane of the test-object is 125 cm. and the diameter of the circular field of the latter 3.5 cm. The distance from the diaphragm to the pupil of the eye is perhaps 1.5 cm. so that the divergence of the light-rays between the artificial and natural pupils is in the neighborhood of $\frac{3.5}{125} \times 1.5$ cm. or about 0.4 mm. This applies to the light from the entire face of the test object. The light from any point on its surface forms a pencil whose divergence after leaving the diaphragm, nearly 125 cm. away, is proportional to the diameter of the latter and negligible in the short distance remaining before it enters the pupil of the eye. The diaphragm is then the aperture from which the theoretical resolving power of the system is to be computed, and there is assurance that the

portion of cornea and lens used does not exceed the diaphragm by more than 0.4 mm. in diameter.

In order that the diaphragm should always be centered in the visual axis certain precautions are used. The test-object is viewed against a milk glass illuminated from behind by a lamp on an enclosed track. The room is kept dark except for an electric wall light back of the observer, which by means of a rheostat he can dim to a point such that the surroundings of the bright test field are just visible. The opening of the diaphragm, close to the eye, then appears as a blurred dim circle, and the observer's head, supported by a forehead-and-chin support is adjusted so that the test field appears exactly in the center of the circle. The wall light is kept lit during the experiment so that the observer always has a check on the position of his eye. The right eye is always used, vision by the other being cut off by a small black cloth hanging before it.

Six diaphragms are used of openings 1, 1.4, 2, 2.8, 4, 5.6 mm.[2] in diameter respectively, each having therefore approximately double the area of the next smaller. It is obvious that a change in the size of the diaphragm changes the amount of light entering the eye, and consequently the brightness of the image. Further, it is well known that simple increase in the brightness of a test-

[2] In order to show that the artificial pupil was always the limiting aperture, photographs of the eyes used have been since taken by flash-light. A millimeter scale was placed beside the eye and its photograph used to measure that of the pupil. The test-object was set at its highest brightness (189 candles per square meter) and the wall light used as in the experiments. Two photographs of each eye were taken, one as soon as possible after entering the experiment-room, and another on another day, after 20 minutes spent in manipulating and viewing the test-object as in the experiment. The results gave the following pupillary diameters.

	At once	*After 20 minutes*
C	6.0–6.2	6.0–6.2
G	6.5–6.75	6.1
J	6.8–7.0	6.5–6.7

The uncertainties in this method of measurement are indicated. The pupil showed a tendency to take a smaller diameter in two cases as the eye dark-adapted. Evidently the conditions under which these measurements were taken (without the artificial pupil) let more light into the eye than any experimental condition used, and even the largest artificial pupil (5.6 mm.) was unquestionably the limiting aperture in the experiments.

object increases the power of the retina in the perception of its detail. It is, therefore, necessary if we wish to gain knowledge of the character of the retinal image under varying apertures to eliminate differences of brightness of the retinal image. This is accomplished by a method about to be described as one detail of the experimental procedure.

Procedure

The 5.6 diaphragm is put in place. The observer, seated properly, begins to operate the test-object by means of the hand-wheel, and at a word from him the drum of the kymograph is started. He keeps the lines of the test-object as nearly as possible at the point of disappearance (or reappearance) and at the end of a minute the kymograph is stopped, the next smaller diaphragm substituted, a one-minute run taken and so on. When the smallest diaphragm is reached, a second run is taken with it, after a brief rest, and the series so repeated in reverse order.

Before each run, the lamp illuminating the test object is moved to such a point that the change in the amount of light entering the eye due to the change in the area of the diaphragm is balanced by an inversely proportional change in the brightness of the test-object. For example in changing from the 1.4 mm. diaphragm to the 1·mm. the illumination is increased as $(1)^2$ to $(1.4)^2$ or doubled, so that the retinal image of the test-object remains of the same brightness. This is practically accomplished by determining photometrically the positions of the lamp on the track which give illuminations as 1, 2, 4, 8, 16 and 32 and using each with its proper diaphragm.

The object of this detail of the procedure is to equalize all factors on which vision may depend other than those inherent in the refractive apparatus of the eye and dependent in magnitude upon the size of the aperture. Better vision results from simple increase in the amount of light, and if this were not compensated, might result from it alone when the aperture is increased. By diminishing the illumination of the test-object the retinal image is in each case brought to equal mean illumination, and a

difference in visual acuity can under such conditions be due only to unequal perfectness of the respective images.

With each observer, two series were taken as described, and two more without compensatory change of the illumination with the lamp at the nearest and farthest positions respectively. This latter gave the test field an average brightness of 5.92 candles per square meter measured photometrically, and the former consequently 32 times this or 189 candles per square meter.[3]

Results and Discussion

The detailed results from the three observers are given in the table together with the mean of the results for each set of conditions. These latter are embodied in the curves in figure 1.

From the table it will be seen that of the three observers, C[4] yielded (with few exceptions) the lowest values for visual acuity and J the highest while G stood between the two. It is further to be noted that every series of results showed a maximum value at some particular diameter of aperture, and that diameters greater and less than this optimum in the same series both gave lower results. This is to be attributed to the predominance of the effects of optical defects in the case of the larger diaphragm, and of the diffraction effect in the case of the diaphragm smaller than the optimum. In support of this it is to be noted that the mean variations of the individual results of the three observers from the average of the three are less under like external conditions for the small apertures, and increase somewhat irregularly with increase in the latter. In going from larger to smaller apertures the individual differences in the various eyes, in respect to their refractive regularity, become less and less important

[3] A perfectly diffusing surface which reflects all the light cast upon it has a brightness of 1 candle per square meter when the illumination upon it is π meter-candles. The two brightnesses given would then be matched by such a surface under illuminations of 18.6 and 593 meter-candles respectively. Any other surface which absorbs a part of the light would require a correspondingly higher illumination upon it to present an equal brightness.

[4] C wore correcting glass, −0.25 D sph. + 1.75 D Cyl. Axis at 90°. The other observers required no correction.

and the diffraction effect, dependent alone on the size of the artificial pupil used and hence equal for all the eyes, increases and finally predominates in determining the limit of visual acuity.

The Relation between Visual Acuity and Size of Artificial Pupil

BRIGHTNESS CANDLES PER SQUARE METER	OBSERVER	APERTURE MILLIMETERS											
		5.6	M.V.	4	M.V.	2.8	M.V.	2	M.V.	1.4	M.V.	1	M.V.
I	C	5.06	0.27	5.28	0.11	5.44	0.25	*5.85*	0.14	4.88	0.21	3.95	0.04
	G	5.62	0.72	*5.83*	0.31	*5.69*	0.27	*5.81*	0.27	4.89	0.29	3.89	0.10
Compensated	J	6.70	0.16	*7.06*	0.21	*6.99*	0.09	6.48	0.14	5.31	0.06	4.09	0.04
	Mean	5.79		*6.06*		*6.04*		*6.05*		5.03		3.98	
	M. V.	0.60		0.67		0.63		0.29		0.19		0.08	
II	C	5.42	0.11	6.15	0.03	*6.44*	0.10	6.23	0.15	5.24	0.04	4.02	0.05
189	G	*7.08*	0.04	*7.14*	0.02	6.70	0.28	6.49	0.12	5.29	0.09	4.08	0.02
	J	*8.10*	0.18	*8.25*	0.08	8.06	0.08	7.17	0.21	5.44	0.06	3.98	0.03
	Mean	6.87		*7.18*		7.07		6.63		5.32		4.03	
	M. V.	0.96		0.71		0.66		0.36		0.08		0.04	
III	C	4.75	0.17	5.01	0.29	*5.35*	0.15	4.32	0.16	4.19	0.30	3.41	0.09
5.92	G	5.34	0.29	*5.77*	0.21	*5.76*	0.16	5.26	0.39	4.56	0.08	3.61	0.06
	J	7.10	0.11	*7.48*	0.05	6.90	0.27	6.13	0.39	4.57	0.28	3.55	0.02
	Mean	5.73		*6.09*		6.00		5.24		4.44		3.52	
	M. V.	0.91		0.93		0.60		0.61		0.17		0.08	

The values for visual acuity here given are in terms of the instrument reading. They may be translated into Snellen units by multiplying by 0.231.[5]

The results in Division I of the table are the means of four one-minute runs for each observer, in Divisions II and III two. The columns headed "M. V." give the mean variations of the separate results from the mean for each case. Similarly, the final results from the separate observers under identical conditions have been averaged and their mean variations given in the horizontal rows also designated "M. V."

These latter, therefore, measure the divergence of results between observers, the former the divergence of the individual observer's results.

It is interesting in this connection to compute the resolving power of the eye with the smallest diaphragm used and compare

[5] This comparison is to be qualified, owing to the fact that in the test-object used the bright and dark lines are only maxima and minima in brightness, the transition from the one to the other being a gradual one. In the case of the usual test-characters the transition is of course abrupt, between the low uniform brightness of the black and the high uniform brightness of the white parts of the test-object.

it with the results actually obtained. From the formula $\theta = 1.22\frac{\lambda}{D}$ when $\lambda = 0.00057$ mm. (the brightest part of the spectrum) and D = 1 mm. θ (expressed in minutes) = 2.39. The angular separation of the bright lines, when the test object is set to read 4.00 is 2.16 minutes—showing the observers' eyes to give

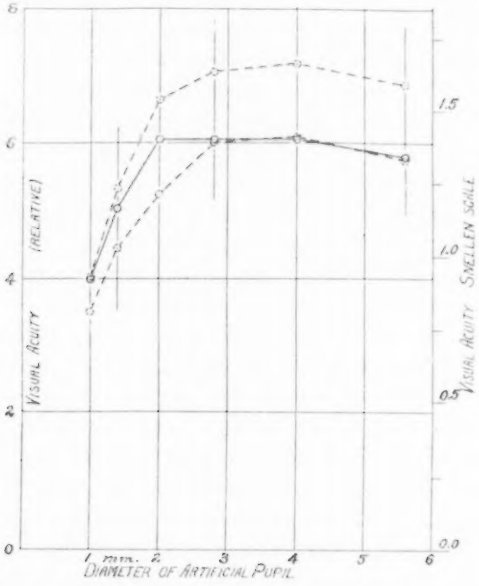

Fig. 1. The values plotted are the means for three observers. The solid line represents the values obtained with brightness compensated, the upper dotted line with constant brightness of 189 candles per square meter, the lower 5.92.

somewhat better vision than is called for by the formula. This is possibly because the latter is based on the smallest resolvable separation of two simple bright points, while the present experimental conditions have to do with an indefinite series of parallel bright lines which might favor the eye under an equal angular separation of the bright elements.

The optimal values for the pupillary diameter are indicated

by italicization of the results in the table. It will be seen that as a general rule, the eye which under equal conditions gives the best values for visual acuity, demands a larger pupil, other factors being equalized, than does an inferior eye to give the best result of which it is capable. Since *caeteris paribus* visual acuity increases with increase of brightness of the test-object, it is not surprising that where the brightness of the retinal image is allowed to increase with diameter of pupil (Divisions II and III of the table) the size of aperture for optimum vision is somewhat larger than it is when the test-object brightness is so compensated as to equalize the brightness of the retinal image (Division I).

Uhthoff obtained results mentioned above, with which the present ones are in agreement. On the other hand, Hummelsheim found increase in vision with decrease in pupillary diameter without exception. The fact that he did not compensate the illumination to obtain equal brightness of retinal images does not account for the discrepancy. As far as can be gathered from his work in the case of the observer showing the lowest visual an artificially contracted pupil (1.5 mm.), was 2.3 at 200 mk. illumination, and with the natural pupil (4 to 4.25 mm. at this illumination) 2.0. The present results show nothing but decrease of visual acuity with decrease in aperture between these limits. Attention is called here to the additional fact that in the present work visual acuity shows an absolutely lower value within this range, at brightnesses both above and below that just mentioned as used by Hummelsheim. If these latter were plotted in figure 1 the points would fall on the 4 mm. ordinate almost 40 per cent again as high as the present maximum (Snellen 1.68) and at a higher point as the zero ordinate is approached. This curve would be for a brightness of test-object intermediate between the two corresponding to the two dotted curves in the figure. This wide discrepancy is difficult to account for. It is to be borne in mind that the results of Uhthoff, obtained through technique similar to the writer's agree well with the latter. There are three possible reasons for the difference.

1. The fact that an artificial pupil allows a certain amount of divergence of the rays after passing through it, so that the area of the refracting surfaces of the eye so called into use is

somewhat in excess of the area of the artificial pupil. This would give the refractive errors of the eye greater weight than the case of a natural pupil of the same apparent diameter. However the difference due to this, as previously pointed out, is quite small.

2. The possibility in the present work that unknown to the observer his eye became, during the observation, somewhat decentered with reference to the axis of the system, so that in the case of the small diaphragm eccentric portions of the refracting surfaces of the eye came exclusively into play. This would tend to increase at least the chromatic error. There stands, however, the fact of the precautions used against this, and the further fact of the close agreement of all results obtained under like conditions in the present work (Divisions I and II of table, under 1 mm. aperture). This last point seems to rule out any error which could fluctuate enough to cause this wide discrepancy.

And 3, the fact that while Hummelsheim had his observers view the test character on a white card, with surroundings also illuminated and the immediate surroundings equal in brightness to the card, Uhthoff and the present writer used a test object on a background chiefly dark. Such a difference in external conditions undoubtedly affects the condition of the retina, although previous work (9) (10) has shown only a slight difference in visual acuity due to dark or bright surroundings, unless the latter are increased to a brightness considerably in excess of that of the test-object. This was not the case in Hummelsheim's method, and further, such a condition (excessively bright surroundings) always results in lower visual acuity, not higher.

The disagreement between his results and the present ones still wants explanation.

Summary of Conclusions

By the use of circular diaphragms before the eye it is shown that an aperture for optimal visual acuity exists somewhere between the limits 1 and 5.6 mm. for brightness of test-object from 5.9 and 189 candles per square meter.

When the illumination of the test object is compensated for the size of aperture to give equally bright images upon the retina

the optimum is somewhat less than when constant illumination is used and the brightness of the retinal image varies with the pupillary area. In the former case it falls at 2 to 4 mm., in the latter at 4 mm. on the average. Similar differences are plainly shown in the case of each of the observers.

Those observers showing on the whole better vision show also a larger optimal pupil.

With an aperture of 1 mm. diameter the several observers give almost identical results, which agree closely with the value of visual acuity calculated from the physical formula for the resolving power of the eye. Above this diameter the refractive errors of the eye and possibly also the limiting capacity of the retina itself come into play, and visual acuity, although at first increasing with increase of aperture, always fails to keep pace with the value computed from the formula.

The optimal pupil corresponds on the whole with the size of pupil accepted as normal for all except extreme conditions, namely, 2.8 to 4 mm. From this lower limit up to 5.6 mm. the variations in visual acuity with size of aperture are not large enough to be of practical consequence.

The writer wishes in conclusion to express the thanks due to his colleague, Dr. H. M. Johnson and to Mr. George Hathaway, technical assistant, for their coöperation and indispensable services as observers in this work, and to Mr. Albert Scheel and Mr. Roy Kerslake for the labor of computing the results.

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THE EFFECT OF REPEATED INJECTIONS OF PITUITRINE ON MILK SECRETION

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It is well known that the subcutaneous, intramuscular or intravenous injection of pituitary extract into lactating animals, man included, leads to an immediate increase in the amount of milk secreted by the mammary gland, abnormally rich in fat. This fact would at once suggest a practical method of increasing the output of milk in dairy cattle or milch goats, but it is found that there is a diminution in the yield at the next milking period, so that for the twenty-four hours the total quantity of milk or cream is not increased. There would appear to be another reason against the habitual use of pituitrine as a galactogogue, viz., that immunity to its action on the mammary gland is established. This, at any rate, we find to be the case in some of the goats with which we have been experimenting, and the observation we deem worthy of being put on record.

In our experiments on milk secretion in the goat we have used one animal for pituitary injection, at various periods extending over one year, and have found that the extract appears to become less and less effective as time goes on. This goat, when she came into our possession (October 31, 1913), was in a late stage of lactation, having given birth to her last kid in the end of March or beginning of April of the same year. On January 27, 1914, another kid was born. Neither of the kids was suckling when we made our experiments.

In the subjoined table (1) will be seen the effect of the injections (2 to 3 cc. of Parke, Davis and Company's pituitrine) on the different dates. The goat was milked dry immediately before

injection and again fifteen minutes after. The last section of the table (beginning July 14, 1914) gives the quantities obtained, under the same conditions, when no injection was given, i.e., the animal was milked dry and then fifteen minutes later milked dry again.

TABLE 1

Goat I

1913	MILK IN CC. BEFORE INJECTION	MILK IN CC. AFTER INJECTION	INCREASE PER CENT	FAT PER CENT BEFORE INJECTION	FAT PER CENT AFTER INJECTION
Nov. 27	50	40	80	12.9	18.0
Dec. 6	33	18	55	6.7	12.6
Dec. 7	35	18	51	6.3	10.2
Kid born January 27, 1914					
1914					
July 13	40	5	12	7.8	9.6
Oct. 1	28	2	7	8.3	
Oct. 16	25	4	16	9.5	
Oct. 20	20	2	10	9.7	
Control milkings without injection					
1914					
July 14	45	4	9	7.8	10.0
Oct. 2	30	1	3	8.2	
Oct. 3	25	1	4		
Oct. 17	20	1	5	8.6	
Oct. 19	20	2	10	9.1	

The next table (2) shows the results of the injection in the same animal (Goat I) made under somewhat different conditions. At 5 p.m. the pituitrine was injected without previous milking and fifteen minutes later the udder was milked dry. The amount of milk yielded at the same hour on the evening before, without injection, is taken as the control.

In another series of experiments, made for a different purpose, we were able to compare the effects on two goats, one (Goat I) which had been used in this work for several months and the other (Goat II) which had been in our possession for only a few days. Goat II, an old animal, was giving more milk at the time than Goat I.

Each was milked dry at 9 a.m. and again at 4 p.m.; at 6 p.m. the pituitrine was injected and fifteen minutes later the udder was emptied again. In table 3 the first column shows the milk in cubic centimeters yielded at 4 p.m. and the second at 6.15 p.m., after injection, for each goat.

Comparing the numbers on the control days with those on the days of injection, in the case of Goat I there is very little difference, whereas in Goat II the contrast is marked. On June 5 only 20 cc. of milk had accumulated in the gland between the hours of 4 p.m. and 6 p.m., while on the day following, in the

TABLE 2

Goat I

1913		AMOUNT OF MILK IN CC.	INCREASE IN CC.	INCREASE PER CENT	PERCENTAGE OF FAT
Nov. 19.....	No injection..	58			
Nov. 20	Injection.....	88	30	52	
Nov. 23	No injection..	75			8.9
Nov. 24	Injection.....	90	15	20	11.4
	Second lactation period				
1914					
June 3	No injection..	40			7.4
June 4	Injection.....	40	0	0	8.4
July 9	No injection..	40			8.0
July 10	Injection.....	40	0	0	7.7

same two-hour period, under the influence of pituitrine, 100 cc. was obtained.

In a third goat the same effect is apparent. This was a young animal in her first lactation period. A kid was born on June 26, 1914, and killed by dogs on July 1. The mother came into our possession on July 3 after which she was milked by hand regularly, morning and evening.

On the dates given in table 4 this animal was milked dry at 6 p.m., injected, and again milked 15 minutes later. On alternate days the same procedure was adopted except that no pituitrine was given; the results are recorded in the second part of the table.

TABLE 3

1914	GOAT I				GOAT II			
	Milk in cc.		Fat per cent		Milk in cc.		Fat per cent	
	4 p.m.	6.15 p.m.	4 p.m.	6.15 p.m.	4 p.m.	6.15 p.m.	4 p.m.	6.15 p.m.
June 6	40	11	6.5		90	100	7.8	10.5
June 8	35	15	9.2	8.0	75	90	6.6	11.9
June 10	25	15	7.8	6.4	80	70	8.0	10.0
Control on days when no pituitrine was given								
June 5	45	8	7.6	5.4	100	20	5.8	7.3
June 7	45	14	8.8	8.2	95	22	5.9	5.8
June 11	40	15	8.3	8.4	85	25	6.5	4.9

A glance at the figures given above will show that the mammary gland appears to become less and less susceptible to the influence of pituitary extract the longer the administration is continued. This applies both to the quantity of milk yielded and to its fat content. The effect is best seen in the case of Goat I but it is also apparent in Goat III.

TABLE 4

Goat III

1914	MILK IN CC.		INCREASE PER CENT	FAT PER CENT	
	Before injection	After injection		Before injection	After injection
July 10	110	60	55	8.0	9.6
July 13	170	35	21	6.8	9.8
Oct. 1	70	10	14	8.7	10.0
Oct. 16	70	10	14	7.7	9.8
Oct. 20	70	7	10	9.7	8.0
Control milkings without injection					
July 14	200	5	2.5	7.2	10.8
Oct. 2	70	5	7	6.4	
Oct. 3	55	6	10	6.4	10.0
Oct. 17	70	5	7	8.2	10.4
Oct. 19	75	4	5	9.0	

Hammond finds that a goat, in the early stages of lactation, is more sensitive to small doses than one in a later stage, but our doses were all maximal, that is, 2 cc. or more. The stage of lactation cannot be the sole influence since in tables 1 and 2 both sets of observations were made about eight months after parturition, but in different lactation periods.

If it be a fact that immunity is established, then the pituitary secretion cannot act as a stimulant for the mammary gland in normal conditions.

SUMMARY

The administration of pituitary extract, by intravenous, intramuscular or subcutaneous injection, to a lactating animal leads to a marked increase in the quantity of milk secreted and also in its fat content. In the goat, if the injection be continued at intervals over a prolonged period—several months—immunity to its action on the mammary glands appears to be established both in regard to the amount of milk yielded and the percentage of fat it contains.